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ANALYSIS OF THE EXPRESSION OF CYP3A4 AND CYP3A5 mRNAs IN HUMAN LIVER BY BRANCHED DNA (bDNA) ASSAY

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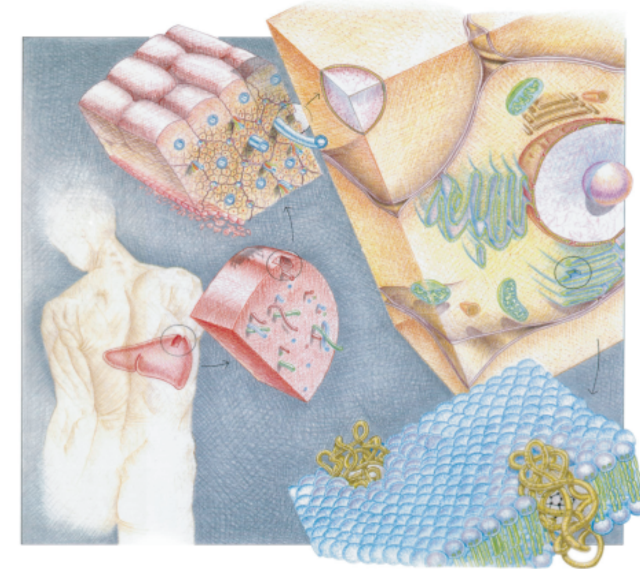

Abstract:

Cytochrome P450 (CYP) enzymes of the CYP3A subfamily play a central role in the oxidative metabolism of xenobiotics in humans. The CYP3A-mediated oxidation of drugs in human liver microsomes is highly variable among individuals. The individual contribution of CYP3A4 and CYP3A5 to hepatic drug metabolism is just beginning to be understood. In the past, extensive sequence identity of the genes and overlapping substrate specificities made progress in this area slow. The aims of this study were

1) to examine whether the variation in level of CYP3A4 and CYP3A5 mRNAs can be semi-quantitatively analyzed with the branched DNA (bDNA) signal amplification assay using gene-specific probe sets,
2) to characterize their levels in tissues in a human liver bank, and
3) to evaluate the inducibility of CYP3A4 and CYP3A5 mRNA in human hepatocyte cultures.

Sequence differences between CYP 3A4 and 3A5 mRNAs were identified and used to develop gene-specific bDNA probe sets. The specificity of the probe sets was demonstrated with E. coli cells cDNA-expressing CYP3A4 or CYP3A5. CYP3A4 mRNA was detected in all livers examined (48) and varied over 700-fold among individuals. CYP3A5 mRNA was also present in all tissues examined but varied only 11-fold. In general, CYP3A4 mRNA levels surpassed those of CYP3A5 (ratio 8:1). However, we identified 12 livers (25%) in which there were higher levels of CYP3A5. These livers all had very low levels of CYP3A4 mRNA. The expression of CYP3A4 mRNA and microsomal testosterone 6β-hydroxylase activity, as well as the levels of the two mRNAs correlated poorly, r^2 = 0.39 and 0.40, respectively. Rifampin, a PXR-mediated inducer of CYP3A4, increased the level of CYP 3A4, but not CYP3A5 mRNA in human hepatocyte cultures.

This study demonstrates that specific bDNA probe sets can be designed to measure expression of highly homologous mRNAs and that they provide a means for studying the differential regulation of CYP3A genes. We also demonstrated that, in a quarter of human livers CYP3A5 mRNA levels exceed those of CYP3A4.

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Introduction

Cytochrome P450 (CYP) CYP3A enzymes play a central role in the oxidative metabolism of xenobiotics in humans. The most abundant CYP3A enzyme, CYP3A4, is highly variable among individuals, which is also true of the minor CYP3A enzymes, namely CYP3A5, 3A7 and 3A43. The expression of the CYP3A genes involves nuclear receptors, which are activated by certain drugs and other xenobiotics, as well as, tissue and age-specific factors. Since the overlapping substrate specificity of the CYP3A enzymes makes it difficult to characterize their abundance based on marker substrate activity, the regulation of the CYP3A enzymes has been studied at the mRNA and or protein level. The aim of this project was to develop a gene-specific method based on branched DNA (bDNA) technology to evaluate the expression of CYP3A4 and CYP3A5 mRNAs. Branched DNA probe sets for CYP3A4 and 3A5 were synthesized and shown to be specific for their respective gene. The probes were used to characterize the abundance of the mRNA in human livers and primary hepatocytes treated with rifampin and phenobarbital. The results of this study demonstrate high variability of CYP3A4 and 3A5 mRNAs and differential regulation of the two genes.

Materials and Methods

Areas of sequence differences between CYP3A4 and 3A5 were identified that allowed gene-specific bDNA probes sets to be developed (see Table 1, and Reference 1). The probe sets are identical with respect to the nucleotide sequence and number of Capture Extenders (CE), Label Extenders (LE) and Blockers (BL), except for 20 base pairs dispersed along the CEs. The specificity of each probe set was evaluated with cell lysates from bacteria transfected with cDNAs expressing either CYP3A4 or CYP3A5. The lysates were mixed in various proportions, as indicated on Fig. 1 and, assayed with QuantiGene™ Signal Amplification High Volume Kit (Bayer Corporation) as recommended by manufacturer. Human tissues and freshly plated hepatocytes were prepared as described in References 2 and 3.

Table 1. Features and Specificity of the bDNA Probe Sets for Human CYP3A Enzymes

Probe set designation and GenBank #	Size of the probe set's target, bp	Number of probe set components			Specificity of capture extenders
		CE	LE	BL	
CYP3A4 J04449	766	3	15	16	CE 3.26: CYP3A4 CE 1487.1507: CYP3A4, CYP3A7 CE 1622.1647: CYP3A4, CYP3A7
CYP3A5 J04813	766	3	15	16	CE 3.26: CYP3A5 CE 1487.1507: CYP3A5 CE 1622.1647: CYP3A5

Results

Figure 1: Specificity of CYP3A4 and CYP3A5 probes

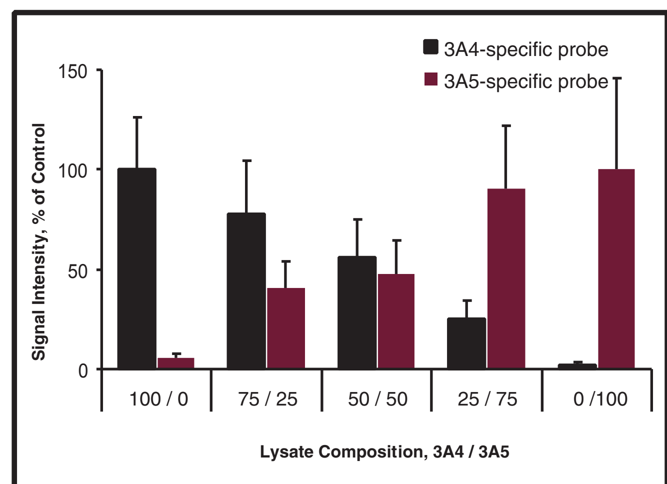


Figure 2: Relation between CYP3A4 mRNA content and testosterone 6β-hydroxylation in 48 human livers

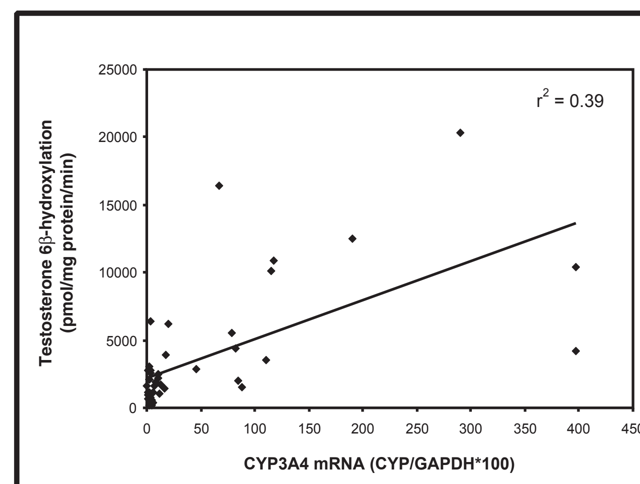


Figure 3: Relation between CYP3A4 and CYP3A5 mRNA content in 48 human livers

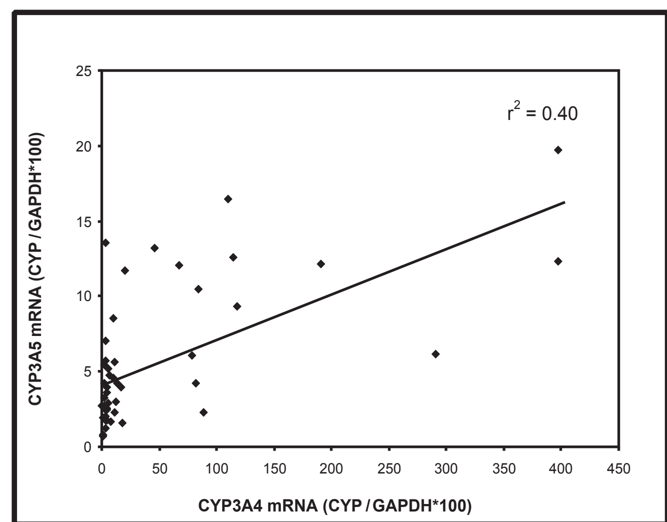
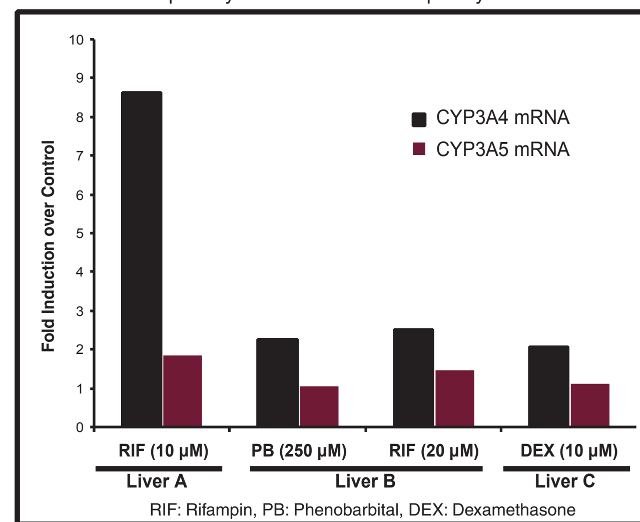


Figure 4: Inducibility of CYP3A4 and CYP3A5 mRNA in primary cultures of human hepatocytes



RIF: Rifampin, PB: Phenobarbital, DEX: Dexamethasone

The bDNA probes were demonstrated to be specific for 3A4 and 3A5, as shown in Fig. 1.

The gene-specific probes were used to evaluate the levels of CYP3A4 and 3A5 in 48 human livers. All tissues contained both mRNAs but their levels were highly variable. The levels of 3A4 and 3A5 mRNA varied 700 and 11 fold, respectively. In general, CYP3A4 mRNA levels surpassed those of CYP3A5 by an 8:1 margin. However, 25% of the livers had CYP3A5 levels that exceeded those of 3A4 mRNA. All these livers had very low levels of CYP3A4 mRNA (below 5% of average) and reduced level of microsomal testosterone 6β-hydroxylase activity (51% of average).

The levels of CYP3A4 mRNA in the 48 samples of human liver correlated poorly with microsomal testosterone 6β-hydroxylase activity ($r^2 = 0.39$, Fig. 2). The correlation between the levels of the two mRNAs was also weak ($r^2 = 0.40$, Fig. 3).

Treatment of two preparations of primary human hepatocytes with 10 and 20 μM rifampin caused an 8.6- and 2.5- fold induction of CYP3A4 mRNA, respectively, whereas CYP3A5 mRNA was induced less than 2-fold in both cases (Fig. 4, livers A and B). In one of these preparations, 250 μM phenobarbital caused a 2.3-fold induction of CYP3A4 mRNA but no induction of 3A5 mRNA. Treatment of one preparation of primary human hepatocytes with 10 μM dexamethasone caused a 2.1-fold induction of CYP3A4 mRNA but no induction of 3A5 mRNA (Fig. 4, liver C).

Conclusions

1. Branched DNA probe sets developed for analysis of CYP3A4 and CYP3A5 are gene-specific despite a high degree of sequence identity between the mRNAs.
2. Expression of CYP3A4 and 3A5 mRNAs is highly variable in human livers. The level of CYP3A4 mRNA correlates poorly with microsomal testosterone 6β-hydroxylase activity, even though CYP3A4 is the major contributor to this reaction. This suggests, but not prove, that regulatory mechanisms other than transcription play a significant role in the expression of CYP3A4.
3. Preliminary results suggest that inducers of CYP3A4 mRNA cause little or no upregulation of CYP3A5 mRNA regardless of whether the induction is mediated primary by PXR (rifampin) or CAR (phenobarbital).

References

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