Short Communication

Sequences of Intestinal and Hepatic Cytochrome P450 3A4 cDNAs are Identical

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ABSTRACT:

Cytochrome P450 (CYP or P450) 3A4 is known to be the major P450 expressed in the liver. More recently, CYP3A4 was also shown to be the major P450 in the intestine, where it plays an important role in the metabolism of some orally administered drugs. However, studies examining the catalytic properties of CYP3A4 have been largely based on the use of CYP3A4 enzyme obtained from liver or recombinant protein expressed from hepatic cDNA. To investigate whether intestinal and hepatic CYP3A4 enzymes are identical, we determined the sequences of intestinal CYP3A4 cDNAs. Compared with the published sequence for hepatic CYP3A4, we found a single base pair difference in the 3' untranslated region of intestinal cDNA from three individuals. We found this same base pair difference in cDNA obtained from the livers of three additional subjects. We conclude that hepatic and intestinal CYP3A4 cDNAs are identical and that the proteins expressed in these two tissues are therefore likely to be the same.

P450 enzymes are important in the oxidative, peroxidative, and reductive metabolism of a large number of endogenous and exogenous compounds. CYP3A4 is the most abundant P450 enzyme in the liver and appears to be the P450 most often involved in drug metabolism (Shimada et al., 1994; Wacher et al., 1995). CYP3A4 is also present in the epithelial cells (enterocytes) lining the small bowel, where it is again the most abundant P450 (Kolars et al., 1992). Enterocyte CYP3A4 has been shown to contribute substantially to the "first-pass" metabolism of some orally administered CYP3A4 substrates (Kolars et al., 1991). Substrates for CYP3A4 include many commonly used medications, such as steroids, calcium channel blockers, immunosuppressive agents, imidazole antimycotics, and macrolide antibiotics (Wacher et al., 1995).

Researchers in industrial and academic laboratories routinely study CYP3A4 activity with liver-derived materials such as human liver slices, cultured human hepatocytes, human hepatic microsomes, and recombinant CYP3A4 derived from hepatic CYP3A4 cDNA (Chiu, 1993). It has been assumed that observations regarding CYP3A4 catalytic activity in liver-derived systems are directly applicable to CYP3A4 in the intestine. In support of this, we and others have shown that the migration patterns for intestinal and hepatic CYP3A4 in sodium dodecyl sulfate-polyacrylamide gels are indistinguishable that the migration patterns for intestinal and hepatic CYP3A4 in sodium dodecyl sulfate-polyacrylamide gels are indistinguishable. However, we found no detectable change in the level of hepatic CYP3A4 activity (as measured by the erythromycin breath test) after grapefruit juice ingestion, indicating that the effect of grapefruit juice is selective for intestinal CYP3A4 (Lown et al., 1997a).

In addition, the relative contributions of hepatic and intestinal CYP3A4 to first-pass metabolism have been shown to vary among medications. Whereas hepatic CYP3A4 appears to be the predominant determinant of metabolism for po administered cyclosporin A (Lown et al., 1997b), intestinal CYP3A4 appears to be the major factor determining the bioavailability of po administered felodipine (Lown et al., 1997a).

Finally, catalytic activity differences between hepatic and intestinal CYP3A4 were suggested in a study of cyclosporin A metabolite formation in healthy volunteers (Hebert et al., 1993). In that study, AM1 was found to be the primary cyclosporin A metabolite formed after both iv and po administration of cyclosporin A. When the same individuals were given iv cyclosporin A after pretreatment with rifampin (a potent inducer of CYP3A4 in both liver and intestine), AM1 remained the primary metabolite formed. However, the major metabolite formed after po administration of cyclosporin A changed from AM1 to AM9 after rifampin pretreatment, suggesting catalytic differences between the enzymes induced in the liver and the intestine.

The sequence of CYP3A4 cDNA obtained from hepatic libraries has been extensively examined (Molowa et al., 1986; Beaune et al., 1986; Gonzalez et al., 1988). We hypothesized that organ-specific differences in the coding regions of the mRNAs, perhaps resulting from alternate splicing, could exist and might explain the discrepancies noted between hepatic and intestinal CYP3A4 catalytic activities. We therefore cloned and sequenced human intestinal CYP3A4 cDNA.

We recently showed that the increase in the availability of felodipine observed when it is administered po with grapefruit juice is due, at least in part, to reduction of the enterocyte concentration of CYP3A4 protein, without any accompanying change in intestinal CYP3A4 mRNA levels (Lown et al., 1997a). This is likely the result of mechanism-based inactivation of CYP3A4, with subsequent rapid intracellular degradation of the enzyme (Lown et al., 1997a). However, we found no detectable change in the level of hepatic CYP3A4 activity (as measured by the erythromycin breath test) after grapefruit juice ingestion, indicating that the effect of grapefruit juice is selective for intestinal CYP3A4 (Lown et al., 1997a).

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Abbreviations used are: P450 or CYP, cytochrome P450; PCR, polymerase chain reaction.

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Materials and Methods

Human Tissue Samples. Small-bowel biopsies were obtained from three individuals scheduled to undergo upper intestinal endoscopy for clinical indications. These patients were receiving no medications known to induce or inhibit CYP3A activity, had no known small-bowel disease, and had no contraindications to intestinal biopsies. Liver tissue was obtained from explanted livers from three individuals with end-stage liver disease who were undergoing orthotopic liver transplantation. Written informed consent was obtained from all individuals. Procurement of the tissue samples was approved by the Institutional Review Board at the University of Michigan.

mRNA Isolation. Ten small-bowel biopsies distal to the duodenal bulb were obtained from three patients, immediately homogenized in 400 μl of denaturing solution (4 M guanidine isothiocyanate, 0.5% N-lauroylsarcosine, 25 mM sodium citrate, and 0.7% 2-mercaptoethanol, pH 7.0), and frozen at −80°C. Later, the samples were quickly thawed and the RNA was extracted by the method of Chomczynski and Sacchi (1987). Liver samples were taken from the freshly explanted livers, immediately snap-frozen in liquid nitrogen, and stored at −80°C. At a later time, the samples were thawed and immediately homogenized in denaturing solution for RNA extraction (Chomczynski and Sacchi, 1987).

Cloning and Sequencing of CYP3A4. One microgram of total RNA was reverse-transcribed to cDNA and amplified by PCR, using synthetic oligonucleotide primers specifically designed to amplify three overlapping segments of the CYP3A4 cDNA (fig. 1, segments A, B, and C). These primers do not amplify cDNA from CYP3A5 or CYP3A7, the other members of the CYP3A family (data not shown). The overlapping fragments generated by PCR spanned the hepatic CYP3A4 sequence from position 15 to position 2059 (GenBank accession number M18907) (fig. 1). The amplified DNA fragments were ligated directly into pBluescript (Stratagene, La Jolla, CA). The ligated DNA was transfected into DH5α Escherichia coli, and the plasmid containing the PCR insert was purified using a Magic Miniprep kit (Promega, Madison, WI).

The sequence of each of the three DNA segments was determined by the dideoxy termination method (Sanger et al., 1977), using 22–30-base pair oligonucleotide sequencing primers synthesized in the University of Michigan Molecular Core Facility. All sequencing reactions were repeated twice. All segments were found to differ from the published sequence for hepatic CYP3A4 were recloned and sequenced a total of three times, to differentiate Taq polymerase incorporation errors from actual sequence variations. The sequences from the individual DNA fragments were joined using the GELASSEMBLE function of the Genetics Computer Group software package (Wisconsin Package, version 9.0; Genetics Computer Group, Madison, WI). Comparisons between the hepatic (GenBank accession number M18907) and intestinal CYP3A4 sequences were made by BESTFIT analysis.

Results

Three large fragments of intestinal CYP3A4 cDNA were amplified by PCR of cDNA prepared from small-bowel biopsies obtained from a single individual (patient A) (fig. 1). The fragments were cloned as outlined in Materials and Methods. Oligonucleotide primers were used to sequence overlapping 200–300-base pair regions of the cloned fragments, as illustrated in fig. 1. A consensus sequence was generated using the GELASSEMBLE function of the Genetics Computer Group software. A BESTFIT comparison of the consensus intestinal CYP3A4 sequence with the published hepatic sequence revealed a single base pair difference of a cytosine instead of a thymine at position 1840. This nucleotide difference was confirmed by recloning and sequencing fragment C several times, to rule out reverse transcriptase or Taq polymerase incorporation errors.

To further examine this potential difference, we amplified and cloned fragment C from intestinal RNA from two additional individuals. Sequence analysis revealed that both of these subjects demonstrated a cytosine at position 1840.

To determine whether this sequence difference was unique to the intestine, we amplified and cloned fragment C from hepatic RNA obtained from three patients undergoing liver transplantation. The sequences for all three individuals were identical to the published hepatic CYP3A4 sequence, except that once again there was a cytosine to thymine conversion at position 1840.

Discussion

We began this investigation because there was evidence suggesting that the catalytic activities of CYP3A4 in the intestine and the liver might be different. We hypothesized that this might be due to differences in the mRNA sequence present in intestinal enterocytes, such as splice variants. We did find that there was a single nucleotide difference (thymine instead of cytosine at position 1840) in the 3′ noncoding region of intestinal mRNA obtained from three individuals, compared with the published hepatic CYP3A4 sequence. However, this same nucleotide difference was also found in cDNA prepared from the liver of three additional individuals. It therefore seems likely that this represents a common and previously unrecognized allele of CYP3A4.

Because the nucleotide substitution occurs outside the coding region, it should not result in differences in the amino acid sequence of the protein. It therefore seems reasonable to conclude that the enzymes in intestine and liver are structurally identical. This is consistent with recent observations that the catalytic properties of CYP3A4 in human hepatic and intestinal microsomes are indistinguishable (Paine et al., 1996; Fitzsimmons and Collins, 1997; Kolars et al., 1991). There are a variety of explanations for the previous observations suggesting catalytic differences between intestinal and hepatic CYP3A4. For example, the selective effect of grapefruit juice on intestinal but not hepatic CYP3A4 (Lown et al., 1997a) may reflect higher concentrations of the active ingredients in enterocytes than in hepatocytes, resulting from dilution in portal blood after absorption or from degradation within the intestine.

Differences in the relative contributions of intestinal and hepatic CYP3A4 to the first-pass metabolism of po administered CYP3A4 substrates in vivo are also likely to be due to factors other than differences in the catalytic activities of the enzymes. One possibility is that CYP3A enzymes other than CYP3A4 may be differentially expressed in liver and intestine. For example, CYP3A5 and CYP3A7 are expressed in the intestinal mucosa of some adults. Both of these enzymes metabolize many of the same medications as does CYP3A4, and differences in the expression of these enzymes in liver and intestine could account for organ-specific differences in the metabolism of CYP3A substrates.

Another factor that is likely to affect the first-pass metabolism of CYP3A4 substrates is the interaction between CYP3A4 and P-glycoprotein in enterocytes. P-glycoprotein is a transporter, with broad substrate specificity, that is expressed in the apical membrane of enterocytes, where it functions to transport xenobiotics from the
enterocytes back into the intestinal lumen (Hsing et al., 1992; Saitoh and Aungst, 1995). There is extensive overlap among the substrates for P-glycoprotein and CYP3A4 (Wacher et al., 1995; Schuetz et al., 1995), making it likely that the relative affinity of a substance for P-glycoprotein (transport) vs. CYP3A4 (metabolism) may affect its ultimate fate as it is absorbed from the intestine. Differences in the metabolite profile of po administered cyclosporin A before and after rifampin treatment (Hebert et al., 1993) may also be due to the presence of P-glycoprotein in the intestine. Rifampin induces P-glycoprotein in the human intestine in vivo (Lown et al., 1996), and the primary metabolites of cyclosporin A may be selectively pumped into the lumen of the intestine by P-glycoprotein and not reabsorbed (Gan et al., 1996).

In summary, our data support the conclusion that the CYP3A4 forms expressed in liver and intestine are identical. Hence, it seems likely that observations made using CYP3A4 substrates with human hepatic microsomes or recombinant hepatic CYP3A4 in reconstituted systems should be readily applicable to intestinal metabolism.

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