Expression of CYP3A4 mRNA Is Correlated With CYP3A4 Protein Level and Metabolic Activity in Human Liver

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Abstract. A relatively large amount of human liver tissue was required to determine the exact activity of human hepatic CYP3A. Although, the quantity of available human liver tissue samples is limited. We measured levels of CYP3A4 mRNA by RT-PCR with a radiolabeled primer specific for CYP3A4 and compared mRNA expression with CYP3A4 protein level and metabolic activity in liver. The level of CYP3A4 mRNA was correlated with the levels of CYP3A4 protein and activity. Our results suggest that CYP3A4 protein and activity levels can be predicted from CYP3A4 mRNA levels determined by RT-PCR and using a very small amount of liver tissue.

Keywords: human liver, CYP3A4, mRNA

CYP3A4 is the major form of P450 expressed in the human liver (1, 2) and is responsible for metabolism of many drugs (3). Individual variations in hepatic levels of CYP3A4 protein and activity have been reported (4, 5), and these variations may contribute to adverse drug reactions in some persons. Although it has been suggested that such adverse reactions could be avoided by evaluating individual variations in CYP3A4 levels, each studies are difficult because evaluation of CYP3A4 protein and activity levels requires large amounts of liver tissues. On the other hand, there have been some studies to determine if there is a correlation between CYP3A4 activity and expression of its mRNA; however, these studies produced discrepant results (6, 7). Therefore, we attempted to quantify levels of CYP3A4 mRNA by the reverse transcription-polymerase chain reaction (RT-PCR) with a radiolabeled primer specific for CYP3A4 and compared the transcript data with protein and activity levels to determine if RT-PCR, which requires only small amount of tissue, can be used to predict CYP3A4 status in the liver.

Eighteen liver samples and microsomes from Caucasian transplant donors were supplied by The National Disease Research Interchange through the Non Profit Organization (Chiba). This study was approved by the ethics committee of St. Marianna University Hospital (Kanagawa). Hepatic microsomal protein concentrations were determined by the method of Lowry et al. (8). Testosterone 6β-hydroxylation activity was measured according to the method of Niwa et al. (9). Levels of CYP3A4 protein in human liver microsomes were measured by Western blot analysis with a Human CYP3A4 Western Blotting Kit (Gentest Corp., Woburn, MA, USA) and ECL Plus (Amersham, Piscataway, NJ, USA). Recombinant human CYP3A4 (Gentest) was used as the reference standard.

Total RNA was prepared from liver samples with the RNAgent® Total RNA Isolation System (Promega, Madison, WI, USA). Total RNA (2 μg) was reverse-transcribed with a RETOROsript™ kit (Ambion Inc., Austin, TX, USA) in a final volume of 20 μl at 42°C for 1 h. Primers (sense, 5'-GTG TGG GGC TTT TAT GAT G-3' and antisense, 5'-GGC GAC TTT CTT TCA TCC T-3') were used to amplify a 570-bp fragment of the CYP3A4 cDNA. For amplification of a 445-bp fragment of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA, primers (sense, 5'-GAC AAC TTT GGT ATC GTG GA-3' and antisense, 5'-GAC TAC TTT GTT ATC GTG GA-3') were used. CYP3A4 and GAPDH primers were labeled with T4 polynucleotide kinase and [γ-32P]ATP. Polymerase chain reaction

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(PCR) was carried out with 2 μl of the RT mixture, TaKaRa Taq™ (Takara Shuzo Co., Ltd., Shiga) and 10 pmol of each primer for CYP3A4 and GAPDH. Amplification conditions consisted of preheating at 95°C for 5 min followed by 19 to 41 cycles of denaturation at 95°C for 30 s, annealing at 55°C or 57°C for 1 min, and extension at 72°C for 1.5 min. Reactions were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized by UV transillumination. PCR product bands were excised from the gel and counted in a LSC-3500 scintillation counter (Aloka, Tokyo).

We determined the optimal RT-PCR reaction conditions with two liver samples, one with relatively high protein and activity levels (No. 5: 61.6 pmol/mg protein and 8988 pmol/min per mg protein, respectively) and one with relatively low protein and activity levels (No. 2: 10.0 pmol/mg protein and 1094 pmol/min per mg protein, respectively). As shown in Fig. 1, the level of radioactivity in CYP3A4 PCR products was higher for specimen No. 5 in comparison with No. 2 for less than 25 amplification cycles. This difference disappeared with 31 cycles. Therefore, we used 23 cycles of amplification for CYP3A4 mRNA levels. In contrast, the level of radioactivity in PCR products for GAPDH was not different between specimens No. 5 and No. 2 for any number of cycles. Saturation was observed at more than 35 cycles; thus the optimal number of amplification cycles for GAPDH was determined to be 33, which was within the exponential range.

Large differences of testosterone 6β-hydroxylation, which is a marker of CYP3A activity, were observed in 18 samples of human liver microsomes (Fig. 2A). Western blotting analysis for CYP3A4 protein levels reflected these individual differences (Fig. 2B). CYP3A4 protein level was well correlated with the activity (Fig. 3A, r = 0.94).

CYP3A4 and GAPDH mRNA levels were estimated by RT-PCR with radiolabeled primers. CYP3A4 mRNA levels were normalized to those of GAPDH. The pattern of CYP3A4 mRNA expression was consistent with both protein and activity levels (Fig. 2C). CYP3A4 mRNA expression was correlated with activity (r = 0.95) and with protein level (r = 0.96). These results suggest that individual differences in CYP3A4 protein and activity levels are due to individual differences in CYP3A4 gene expression and that it is possible to predict hepatic CYP3A activity from CYP3A4 mRNA measurements. This would reduce the amount of tissue needed to assess a patient’s CYP3A4 status.

Guengerich et al. (10) reported that the CYP3A4 protein level is correlated with nifedipine oxidation, a marker of CYP3A activity, which is consistent with the results of our present study. Sumida et al. (6) reported that CYP3A4 mRNA levels determined by RT-competitive PCR are correlated with testosterone 6β-hydroxylase activity. These findings are also consistent with those of our study, although they did not examine CYP3A4 protein. Thus, the present study is useful because mRNA, protein, and activity levels were measured simultaneously in the same samples. In contrast, Bork et al. (11) measured mRNA levels by Northern blotting and reported that nifedipine oxidase activity is not significantly correlated with CYP3A4 mRNA levels, and Beaune et al. (7) reported that CYP3A4 mRNA levels measured by Northern blotting are not correlated with nifedipine oxidation activity or CYP3A4 protein levels. These inconsistencies may be due to differences in the methods used to assess mRNA levels or to sample sizes. That is, our methods of RT-PCR combined with
Expression of CYP3A4 mRNA, Protein, and Activity

Fig. 2. Testosterone 6β-hydroxylation activity (A), CYP3A4 protein level (B), and the ratio of CYP3A4 mRNA to GAPDH mRNA (C) in 18 human livers.

Fig. 3. Correlation of testosterone 6β-hydroxylation activity and CYP3A4 mRNA and protein level. A: Correlation of activity and protein level. B: Correlation of mRNA expression and activity. C: Correlation of mRNA and protein levels.
radiolabeled primers possessed both high sensitivity and wide range. Recently, Sy et al. (12) reported a significant correlation between CYP3A4 protein and $V_{\text{max}}$ of testosterone 6β-hydroxylation ($r = 0.82$, $P<0.001$) as well as CYP3A4 protein and its mRNA ($r = 0.52$, $P<0.01$) in more samples ($n = 45$). However, the correlation coefficient ($r = 0.52$) between CYP3A4 protein and its mRNA is smaller than our result ($r = 0.96$), and they do not show the correlation between CYP3A4 mRNA and its activity. As a reason for why the correlation coefficient ($r = 0.52$) in the report of Sy et al. is smaller than the correlation coefficient ($r = 0.96$) that we showed in the present study, the difference in the base sequence of the primer used for measurement of CYP3A4 mRNA can be considered. The sequence of the primer that we designed for CYP3A4 differed in six bases from the sequence of CYP3A5 and differed in five bases from the sequence of CYP3A7. On the other hand, the sequence of the primer that Sy et al. used for CYP3A4 only differed in one base from the sequence of CYP3A5 and was completely in agreement with the sequence of CYP3A7. Therefore, it is thought that the primer that these researchers used for CYP3A4 has lower specificity than the primer that we designed for CYP3A4.

In conclusion, we used RT-PCR with radiolabeled specific primers for CYP3A4 to assess CYP3A4 mRNA expression in 18 human liver specimens and compared mRNA levels with CYP3A4 protein and testosterone 6β-hydroxylation. Significant correlations were observed between CYP3A4 mRNA, protein, and activity levels, indicating that both CYP3A4 protein and activity levels can be predicted from the CYP3A4 mRNA level, which can be determined with only a small amount of human liver tissue.

References

4 Guengerich FP, Turvy CG. Comparison of levels of several human microsomal cytochrome P-450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical liver samples. J Pharmacol Exp Ther. 1991;256:1189–1194.