Cloning of cytochrome P-450 2C9 cDNA from human liver and its expression in CHL cells

Ge-Jian Zhu, Ying-Nian Yu, Xin Li, Yu-Li Qian

INTRODUCTION
Cytochrome P-450 (CYP) is a heme-containing enzyme widely distributed from bacteria to mammals, which catalyzes oxidative or reductive metabolism of a wide variety of substances including endogenous as well as exogenous compounds. Mammalian CYP present in liver microsomes is one of the key enzymatic mechanisms for the metabolism of drugs, pesticides, environmental pollutants, and carcinogens[11]. Mammals possess at least 17 distinct CYP gene families that together code for an estimated 50-60 individual CYP genes in any given species[2]. The human CYP2C subfamily comprises four members, CYP2C8, CYP2C9, CYP2C18 and CYP2C19[3], accounting for 20% of the total CYP in human liver. CYP2C9 is a polymorphic enzyme responsible for the metabolism of a large number of clinically important drugs such as S-warfarin, phenytoin, tolbutamide, torsemide, losartan, fluoxetine, dapsone[4], cyclooxygenase-2 inhibitor: celecoxib[5], nonpeptide angiotensin II receptor antagonist: irbesartan[6] and numerous nonsteroidal anti-inflammatory drugs. It ranks among the most important drug metabolizing enzymes in humans[3].

The combination of gene technology and cell culture technology has provided new opportunities for studying proteins because any gene from any species encoding an protein may be cloned and expressed in bacterial, yeast, or mammalian cells in a defined way[9-18]. This approach to drug metabolism is of particular importance because some of the enzymes are difficult to purify and prepare in sufficient quantities, or expression levels are low, expression is organ-specificity, or the enzyme-product organs are very scarce. These restrictions apply especially for human enzymes. The heterologous expression of the cDNA allows to bypass these restrictions[19]. Human CYP2C9 previously has been expressed in E. coli[20], Salmonella typhimurium[21], yeast[22] COS-1[3], human liver epithelial cell THLE[23], and human hepatic cell line HepG2[24]. Several cell lines stably expressing human CYP1A[25], CYP2B6[26], CYP2A6[27], CYP3A4[28], CYP2C9[29] (in press) and a phase II metabolism enzyme UDP-glucuronosyltransferase, UGT1A9[30] have been established in our laboratory. In this study human CYP2C9 cDNA was amplified with reverse transcription-polymerase chain reaction (RT-PCR), and a transgenic cell line stably expressing CYP2C9 was established.

MATERIALS AND METHODS

Materials
Restriction endonucleases, Moloney murine leukemia virus (MuLV) reverse transcriptase were purchased from MBI Fermentas, Quickspin, Pty Ltd. Taq plus I DNA polymerase, dNTPs, PCR primers, DNA sequence primers and random hexamer primer were supplied by神经纤维瘤病 I型 Corp. DNA sequencing kit was supplied by Perkin-Elmer Corp. The TRIZol reagent, G418, minimum essential media(MEM) and newborn bovine calf sera from Gibco. NADPH from Roche Molecular Biochemicals. Diethyl pyrocarbonate (DEPC), tolbutamide and hydroxytolbutamide were obtained from Sigma Chemical Company. T4 DNA ligase and pGEM-T vector system were supplied by 2.0 (Pharmacia).

RESULTS
The amino acid sequence predicted from the cDNA segment was identical to that of CYP2C9*1, the wild type CYP2C9. However, there were two base differences, i.e. 21T>C, 1146C>T, but the encoding amino acid sequence was the same L7, P382. The S9 fraction of the established cell line metabolizes tolbutamide to hydroxy tolbutamide; tolbutamide hydroxylase activity was found to be 0.465±0.109 μmol·min⁻¹·g⁻¹ S9 protein or 8.62±2.02μmol·min⁻¹·mol⁻¹ CYP, but was undetectable in parental CHL cell.

CONCLUSION: The cDNA of human CYP2C9 was successfully cloned and a cell line of CHL-CYP2C9, efficiently expressing the protein of CYP2C9, was established.
Cloning of human CYP2C9 cDNA from a Chinese human liver

The total RNA was extracted from a surgical specimen of human liver with TRIzol reagent according to the manufacturer’s instructions, and then the first strand of cDNA was reverse transcribed from mRNA. Procedure: 5µg of the total RNA and 2µg of random hexamer primer in deionized DEPC-treated water were denatured at 65°C for 15min, then 4µL 5×reverse transcription buffer, 3µL 10mmol·L⁻¹ dNTP, 1µL M-MuLV reverse transcriptase (200u) and essential deionized DEPC treated water was added to a total volume of 20µL. The reaction was performed at 25°C for 10min, then at 42°C for 1h, and finally at 70°C for 10min to inactivate reverse transcriptase. The reactant then was stored at 4°C. To amplify the human CYP2C9 cDNA by PCR, 2µL of the reactant were mixed with 2µL of 10mmol·L⁻¹ each of dNTPs, 20pmol of PCR primers and 4u of Taq plus 1 DNA polymerase in 1×PCR buffer containing 1.5mmol·L⁻¹ MgCl₂. A total volume of 100µL was reached by adding deionized water. Two specific 32 mer and 28 mer oligonucleotide PCR primers were designed according to the cDNA sequence of CYP2C9 clone 25 reported by Romkes et al. (GenBank accession no. M61855, J05326). The sense primer corresponding to base position 1 to 32 was 5’-GAGAAGTGATCAATGATCTTCTTGTTGCTCT-3’, with a restriction site of Kpn I, and the anti-sense one, corresponding to the base position from 1513 to 1540, was 5’- AGAGAAAGAGAGCTGAGGGACTGAC-3’ with a restriction site of Xho I. The PCR product was performed at 94°C 2min, then 35 cycles of 94°C 60s, 60°C 60s, 72°C 2min, and last 72°C 10min. The product was stored at 4°C. An aliquot of 10µL of the PCR product was subjected to electrophoresis in a 10g·L⁻¹ agarose gel stained with ethidium bromide.

Construction of recombinant pGEM-CYP2C9 and sequencing of CYP2C9 cDNA

The PCR product of about 1.5 kb in length, recovered and purified by electroelution into dialysis bag was ligated with a clone vector, pGEM-T (Promega), by T4 DNA ligase. E.coli DH5α was transformed with the resultant plasmid and the replicated plasmid was harvested from the bacteria screened by ampicillin resistant and blue-white selection with X-gal and IPTG. The cDNA of CYP2C9 cloned in pGEM-T was sequenced on both strands by dideoxy chain termination method marked with BigDye with primers of T7 and SP6 promoters and two specific primers of 5’-TGCCTTGTGGAGTTG-3′ (Promega). Other chemical reagents used were all of analytical purity from commercial sources.

Promega. Other chemical reagents used were all of analytical purity from commercial sources.

RESULTS

Construction of recombinants

The recombinant of pGEM-CYP2C9 cloned in pGEM-T between the promoters of T7 and SP6 was subjected to electrophoresis in a 10g·L⁻¹ agarose gel stained with ethidium bromide. The resulted homogenate was centrifuged at 9000g at 4°C for 20min and the postmitochondrial supernatant (S9) was transferred carefully to a clean tube for assay or storage under -70°C. The protein in S9 was determined by the method described by Lowry et al., with bovine serum albumin as standard. CYP was measured spectrally using the method of Johannesen et al. (22,31).

Tolbutamide hydroxylase assay

The CYP2C9 tolbutamide hydroxylase activity of S9 fraction was determined by high performance liquid chromatography (HPLC). The assay was performed in a total volume of 500µL containing final concentrations of 5nmol·L⁻¹ HEPES (pH 7.4), 1.5mmol·L⁻¹ MgCl₂, 0.1mmol·L⁻¹ EDTA, 0.25mg S9 and 1mmol·L⁻¹ sodium tolbutamide. The reaction was initiated with 0.5mmol·L⁻¹ NADPH and terminated after 60min at 37°C by the addition of 50µL of 4mmol·L⁻¹ HCl. Reaction product was extracted by vortex-mixing of 3mL of water saturated ethyl acetate with the mixture for 2min. The organic layer was collected after centrifugation in a table top centrifuge at 1000g for 5min. After most of the ethyl acetate extract had air-dried, the rest was removed in a heating block at 75°C. The residue was resolubilized in 200µL of methanol, and reaction product, hydroxytolbutamide was then assayed using HPLC by injecting 20µL of the solubilized extract on to a reversed phase column (Shim-pack CLC-ODS 15cmx0.6cm id, 10µm particle size), using 0.5g·L⁻¹ phosphoric acid, pH 2.6, acetonitrile (6:4V/V) as the mobile phase with a flow rate of 1mL·min⁻¹. The column elution was monitored at 230nm, and rates of product formation were determined from standard curves prepared by adding varying amounts of hydroxytolbutamide to incubations conducted without NADPH.
contained the entire coding region, along with 2 bp of the 5’end and 41 bp of the 3’end untranslated region of the CYP2C9 cDNA, respectively.

**Figure 1** Scheme and electrophoresis identification of recombinant pGEM-CYP2C9. A: Scheme of recombinant of pGEM-CYP2C9; B: Electrophoresis identification of recombinant pGEM-CYP2C9; 1: Marker (λ/EcoRI and Hind III); 2: PCR product of CYP2C9(1.54 kb); 3: Recombinant of pGEM-CYP2C9 digested by Kpn I and Xho I; 4: pGEM-T vector

**Establishment of transgenic cell lines with CYP2C9 enzyme activity**

CHL cells were transfected with pREP9-CYP2C9, and selected with G418 (400mg·L⁻¹). The surviving colonies were propagated and a cell line termed CHL-CYP2C9 was established. The tolbutamide hydroxylase activity of CYP2C9 in S9 fraction of CHL-CYP2C9 cells was assayed by HPLC. A typical elution profile of hydroxytolbutamide in extracts is shown (Figure 3). CYP2C9 enzyme activity with tolbutamide was found to be 0.465±0.109µmol·min⁻¹·g⁻¹ S9 protein or 8.62±2.02µmol·min⁻¹·mol⁻¹ CYP (n=3), but none was detectable in parental CHL cells. The CYP content was 57.7nmol·g⁻¹ S9 protein from CHL-CYP2C9 but no detectable CYP was present in CHL cell.

**Figure 2** Scheme and electrophoresis identification of recombinant pREP9-CYP2C9. A: Scheme of pREP9-CYP2C9; B: Electrophoresis identification of recombinant pREP9-CYP2C9; 1: Marker (λ/EcoR I and Hind III); 2: PCR product of CYP2C9(1.54 kb); 3: Recombinant of pREP9-CYP2C9 digested by Kpn I and Xho I; 4: pREP9 vector

**Figure 3** Representative chromatogram of extracts. A Shim-pack CLC-ODS column (15cm×0.6cm i.d.) was used. The mobile phase was constituted with phosphoric acid (pH 2.6), acetonitrile (6:4/V:V) with the flow rate at 1mL·min⁻¹. Hydroxytolbutamide was monitored at 230nm. A: hydroxytolbutamide; B: tolbutamide

**DISCUSSION**

Direct cloning of human CYP cDNAs from cDNA libraries generally has been successful using anti-rodent or anti-human CYP antibodies and rodent CYPcDNA probes. But these cloning procedures are applicable only for the most abundantly expressed CYP mRNAs. Using the RT-PCR to clone low abundance CYPcDNA is a simple and direct method. CYP2C9 mRNA was present in human liver[22], HepG2 cells[23], kidney, testes, adrenal gland, prostate, ovary, duodenum[24], and brain tumors[35]. The pGEM-T vector system possessing single 3’-T overhangs at the insertion site greatly improves the efficiency of ligation of a PCR product into the plasmid by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by Taq Plus I DNA polymerases.

The human CYP2C9 gene is located on chromosome 10q24. Up to date, 12 CYP2C9 alleles have been identified (see: Table 1 and CYP2C9 alleles nomenclature http://www.imm.ki.se/CYPalleles/cyp2c9.htm). CYP2C9*1 is the wild type of human CYP2C9. CYP2C9*2 exhibit a base substitute 430C>T, resulting in a R144C substitution which has been suggested to affect the interaction between the CYP enzyme molecule and the cytochrome P450 reductase[36]; this may explain the slower metabolism of some CYP2C9 substrates such as S-warfarin and tolbutamide[8, 37]. CYP2C9*3 has a base substitute 1075A>C, which leads to an I359L substitute. Takanashi et al[38] expressed the CYP2C9*1 and CYP2C9*3 cDNA in yeast and examined the kinetics of seven individual metabolic reactions by wild-type CYP2C9 and its CYP2C9*3 variant. Their results indicated that the I359L exchange significantly reduces the catalytic activity with all CYP2C9-mediated substrates studied, although the extent of the reduction in activity and kinetic parameters varied between different substrates. Interestingly Kidd et al[39] reported a male Caucasian, homozygous for CYP2C9*3, who poorly metabolized phenytoin and glipizide/tolbutamide. This study establishes that the I359L mutation is responsible for the poor metabolizer phenotype. The CYP2C9*2 and CYP2C9*3 are responsible for the poor metabolizing celecoxib[31], losartan[40], torsemide[41]. CYP2C9*4[42] has a base substitute of 1076T>C,
leading to a I359T substitution. Ieiri et al.[41] evaluated the catalytic activity of three variants (I, L, and T) at codon 359 of CYP2C9 enzymes expressed in a yeast cDNA expression system. The specific catalytic activities were assessed by diclofenac-4'-hydroxylation. The in vitro study revealed that recombinant I359, L359, and T359 (2 batches) exhibited a mean Km of 2.0, 16.5 and (3.8 and 2.9) \( \mu \)mol and Vmax of 12.4, 17.9 and (4.4 and 5.1) nmol-min\(^{-1}\)nmol\(^{-1}\)CYP, respectively. The CYP2C9*5 variant is derived from a 1080C>G transition in exon 7 of CYP2C9 that leads to a D360E substitution in the encoded protein[41]. The CYP2C9*5 variant was found to be expressed in African-Americans with a frequency of approximately 3% in this population group. This variant was expressed in, and purified from, insect cells infected with a recombinant baculovirus. The in vitro data suggest that carriers of the CYP2C9*5 allele would eliminate CYP2C9 substrates at slower rates compared to individuals expressing the wild-type protein[41]. Kidd et al.[40] reported a new CYP2C9 allele (CYP2C9*6) with the deletion of an adenine at base pair 818 of the cDNA. The clearance of phenytoin in this individual is less than that observed in normal patients.

| Allele       | Protein     | Nucleotide changes | Effect   | Enzyme activity           | References |
|--------------|-------------|--------------------|----------|---------------------------|------------|
| CYP2C9*1     | CYP2C9      | None               | Normal   | Normal                    | 3          |
| CYP2C9*2     | CYP2C9      | 430C>T             | R144C    | Decr 36                   | 2          |
| CYP2C9*3     | CYP2C9      | 1075A>C             | I359L    | Decr                      | 4          |
| CYP2C9*4     | CYP2C9      | 1076T>C             | I359T    | 42.43                     | 5          |
| CYP2C9*5     | CYP2C9      | 1080C>G             | D360E    | Decr                      | 6          |
| CYP2C9*6     | CYP2C9      | 818delA            | frame shift Decr | 45                  | 7          |

To express the functional activity of a CYP, a cell evidently must have adequate heme supply, either by intracellular biosynthesis or extracellular provision[41]. CYPs also require other enzymatic components for full activity, including the flavoprotein NADPH oxidoreductase (OR) and, in some cases, cytochrome b. The OR must interact directly with the CYP to transfer the reduced electrons from NADPH. Cytochrome b5 is necessary for increasing electron transfer for certain CYP forms and specific substrates. The CHL is the cell line originally derived from the lung of a newborn female Chinese hamster and has no or very limited activities of CYP enzymes, but has adequate OR and cytochrome b5 levels to support CYP activities.

To achieve high expression levels of CYP2C9, the CYP2C9 cDNA was cloned into the eukaryotic expression vector pREP9, which has previously been used in this laboratory for the expression of human CYP1A1, CYP2B6, CYP2A6, CYP3A4 and UGT1A9 in CHL cells[16,17]. The salient feature of this vector has an Epstein Barr Virus origin of replication and nuclear antigen (EBNA-1) to allow high-copy episomal replication in mammalian cell lines. The Rous sarcoma virus long terminal repeat (RSV LTR) early promoter controls the expression of the CYP2C9 cDNA.

Tolbutamide (1-butyl-1-p-tolyisufonylurea) is an oral hypoglycemic agent which is being used in the treatment of diabetes. In humans it undergoes CYP-catalyzed hydroxylation of the tolyl methyl group which is the initial and rate-limiting reaction followed by further oxidation by cytosolic dehydrogenases yielding carboxyethylbutamide. Overall this pathway accounts for up to 85% of tolbutamide clearance in humans. Evidence that CYP2C9 is solely responsible for tolbutamide hydroxylation is convincing and tolbutamide is widely accepted as a prototypic substrate for the assessment of hepatic CYP2C9 activity, both in vitro and in vivo[48].

We used tolbutamide as a substrate for evaluating the expressing of human CYP2C9 activity in CHL-CYP2C9 cell. The tolbutamide hydroxylase activity was 0.46±0.10µmol-min\(^{-1}\)g\(^{-1}\) S9 protein or 8.62±2.02µmol-min\(^{-1}\)mg\(^{-1}\) CYP. These values are somewhat higher than those obtained with recombinant CYP2C9 purified from E. coli: 4.67±4.96µmol-min\(^{-1}\)nmol\(^{-1}\) CYP[49] or human liver microsomes: 189.0±4.083µmol-min\(^{-1}\)mg\(^{-1}\) microsome[50] and 2.29±3.33µmol-min\(^{-1}\)nmol\(^{-1}\) CYP[48]. This clearly stated that CYP-CYP2C9 expressed the CYP2C9 efficiently and this may be a useful tool for further studies of its enzymatic function and mechanism.

REFERENCES

1. Anzenbacher P, Anzenbacherova E. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 2001;58:737-747
2. Nelson DR. Cytochrome P450 and the individuality of species. Arch Biochem Biophys 1999; 369: 1-10
3. Romkes M, Faletto MB, Blaisdell JA, Raucy JL, Goldstein JA. Cloning and expression of complementary DNAs for multiple members of the human CYP450 ic subfamily. Biochemistry 1991; 30: 3247-3255
4. Winter HR, Wang Y, Unadkat JD. Cytochrome P80 hydroxylation at clinical concentrations of dapsone. Drug Metab Dispos 2000; 28: 865-868
5. Tang C, Shou M, Rushmore TH, Mei Q, Sandhu P, Woolf EJ, Rose MJ, Gelmann A, Greenberg HE, De-Lepeleire I, Van-Hecken A, De-Schepper PJ, Ebel DL, Schwartz JL, Rodrigues AD. In vitro metabolisms of celecoxib, a cyclooxygenase-2 inhibitor, by allelic variant forms of human liver microsomal cytochrome P450 CYP2C9 evolution CYP2C9 genotypes in vivo pharmacokinetics. Pharmacogenetics 2001;11:223-235
6. Tang C, Shou M, Mei Q, Rushmore TH, Rodrigues AD. Major role of human liver microsomal cytochrome P450 2C9 (CYP2C9) in the oxidative metabolism of celecoxib, a novel cyclooxygenase-2 inhibitor. J Pharmcol Exp Ther 2000; 293: 453-459
7. Bourrie M, Meunier V, Berger Y, Fabre G. Role of cytochrome P450 2C9 in ibrexatarn oxidation by human liver microsomes. Drug Metab Dispos 1999; 27: 996-999
8. Miners JO, Bertek DJ, Cytochrome P450 2C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol 1998; 45: 525-538
9. Liu XF, Zou SQ, Qiu FZ. Construction of HCV core gene vector and its expression in cholangiocarcinoma. World J Gastroenterol 2002; 8: 135-138
10. Wu C, Zou QM, Guo H, Zhang XF, Zhang WJ, Lu DS, Mao XH. Expression, purification and immuno-characteristics of recombinant UreB protein of H. pylori. World J Gastroenterol 2001; 7: 389-393
11. Li XJ, Wu JG, Si JL, Guo DW, Xu JP. High-level expression of human calmodulin in E.coli and its effects on cell proliferation. World J Gastroenterol 2000; 6: 598-592
12. Cheng J, Zhong YW, Liu Y, Dong J, Yang JZ, Chen JM. Cloning and sequence analysis of human genomic DNA of augmenter of liver regeneration. World J Gastroenterol 2000; 6: 275-277
13. Guo SP, Ma ZS, Wang WL. Construction of eukaryotic expression vector of HBV x gene. World J Gastroenterol 1999; 5: 351-352
14. Qu S, Li QF, Deng YZ, Zhang JM, Zhang J. Cloning and expression of HLA-B7 gene. World J Gastroenterol 1999; 5: 345-348
15. Liu ZG, Yang JH, An HZ, Wang HY, He FT, Han ZY, Han Y, Wu HP, Xiao B, Fan DM. Cloning and identification of an angiastic molecule IP10/crg-2. World J Gastroenterol 1999; 5: 241-244
16. He Y, Zhou J, Dou KF. Construction of hepatocyte growth factor expression vector and detection of expression in human hepatocytes. Shijie Huaren Xiaohua Zazhi 2001; 9: 1121-1126
17. Pan X, Pan W, Ke CW, Zhang B, Cao GW, Qi ZT. Tetracycline controlled DT/VEGF system gene therapy mediated by adenovirus vector. Shijie Huaren Xiaohua Zazhi 2000; 8: 1121-1126
18. Lu JG, Lin C, Huang ZQ, Wu JS, Fu M, Zhang XY, Liang X, Yao X, Wu M. Inhibitory effects of human cholangiocarcinoma cell line by recombinant adenoviruses P16 with CDDP. Shijie Huaren Xiaohua Zazhi 2000; 8: 641-645
19. Crespi CL, Miller VP. The use of heterologously expressed drug metabolizing enzymes-state of the art and prospects for the future. Pharmacol Therapeutics 1999;84:121-131
20. McGinnity DF, Griffin SJ, Moody GC, Voice M, Hanlon S, Friedberg T, Riley RJ. Rapid characterization of the major drug-metabolizing human...
hepatic cytochrome P-450 enzymes expressed in Escherichia coli. Drug Metab Dispos 1999; 27: 1017-1023
21 Fujita K, Kamataki T. Role of human cytochrome P450(CYP) in the metabolic activation of N-alkylnitrosamines: application of genetically engineered Salmonella typhimurium YG7180 expression each form of CYP together with human NADPH-cytochrome P450 reductase. Mutat Res 2001; 483: 35-41
22 Goldstein JA, Faletto MB, Romkes-Sparks M, Sullivan T, Kitarerwan S, Raucy JL, Lasker JM, Ghanayem BI. Evidence that CYP2C19 is the major (S)-mephenytoin 4'-hydroxylase in humans. Biochemistry 1994; 33: 1743-1750
23 Bort R, Castell JV, Pfeifer A, Gomezlechon MJ, Mace K. High expression of human CYP2C in immortalized human liver epithelial cells. Toxicol in Vitro 1999; 13: 633-638
24 Yoshitomi S, Ikemoto K, Takahashi J, Kobayashi K, Yasumori T, Hosakawa M, Chiba K. CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. Pharmacogenetics 2000; 10: 95-104
25 Wu JM, Dong HT, Cai ZN, Yu YN. Stable expression of human cytochrome CYP2B6 and CYP1A1 in chinese hamster CHL cells: their use in micronuclear assays. Chin Med Sci J 1997; 12:148-155
26 Yan LQ, Yu YN, Zhuge J, Xie HY. Cloning of human cytochrome P450 2A6 cDNA and its expression in mammalian cells. Zhongguo Yaolixue Yu Duxue Zashi 2000; 143:31-35
27 Chen Q, Wu J, Yu Y. Establishment of transgenic cell line CHL-3A4 and its metabolic activation. Zhonghua Yufang Yixue Zashi 1998; 32: 281-284
28 Li X, Yu YN, Zhuge J, Qian YL. Cloning of UGT1A9 cDNA from liver tissues and its expression in CHL cells. World J Gastroenterol 2000; 6: 243-246
29 Sambrook J, Frisch E. Molecular cloning, a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989:6-28-29. 1:63-86, 16:39-40
30 Johannesen KA, DePierre JW. Measurement of cytochrome P-450 in the presence of large amounts of containing hemoglobin and hemoglobinmethhemoglobin. Anal Biochem 1978; 86: 725-732
31 Ho JW, Moody DE Determination of tolbutamide hydroxylation in rat liver microsomes by high-performance liquid chromatography: effect of psychoactive drugs on in vitro activity. Life Sci 1993; 52: 21-28
32 Rodriguez-Antona C, Donato MT, Pareja E, Gomez-Lechon MJ, Castell JV. Cytochrome P-450 mRNA expression in human liver and its relationship with enzyme activity. Arch Biochem Biophys 2001; 390: 308-315
33 Sumida A, Fukuen S, Yamamoto I, Matsuda H, Naohara M, Azuma J. Quantitative analysis of constitutive and inducible CYPs mRNA expression in the HepG2 cell line using reverse transcription-competitive PCR. Biochem Biophys Res Commun 2000; 267: 756-760
34 Klose TS, Blasidell JA, Goldstein JA. Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. J Biochem Mol Toxicol 1999; 13: 289-295
35 Knupfer H, Knupfer MM, Hotfilder M, Preiss R. P450-expression in brain tumors. Oncol Res 1999; 11: 523-528
36 Crespi CL, Miller VP. The R144C change in the CYP2C9*2 allele alters interaction of the cytochrome P450 with NADPH:cytochrome P450 oxidoreductase. Pharmacogenetics 1997; 7:203-210
37 Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1999; 353:717-719
38 Takashashi K, Tainaka H, Kobayashi K, Yasumori T, Hosakawa M, Chiba K. CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. Pharmacogenetics 2000; 10: 95-104
39 Kidd RS, Straughn AB, Meyer MC, Blaisdell J, Goldstein JA, Dalton JT. Pharmacokinetics of chlorphenamine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. Pharmacogenetics 1999; 9: 71-80
40 Yasar U, Tybring G, Hiderstrann M, Oscarson M, Ingelman-Sundberg M, Dahl ML, Eliasson E. Role of CYP2C9 polymorphism in losartan oxidation. Drug Metab Dispos 2001; 29: 1051-1056
41 Miners JO, Coulter S, Birkett DJ, Goldstein JA. Torsemide metabolism by CYP2C9 variants and other human CYP2C subfamily enzymes. Pharmacogenetics 2000; 10: 267-270
42 Imai J, Ieiri I, Mamiya K, Miyahara S, Furunumi H, Nanba E, Yamane M, Fukumaki Y, Ninomiya H, Tashiro N, Higuchi S. Polymorphism of the cytochrome P450 CYP2C in immortalized human liver epithelial cells. World J Gastroenterol 2001; 7:934-938