Overexpression of CYP3A5 attenuates inducibility and activity of CYP3A4 in HepG2 cells

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Abstract. There have been conflicting reports regarding the catalytic role of cytochrome P450 (CYP)3A5, which range from deeming it irrelevant to suggesting it is equally as important as CYP3A4, the most potent and abundant catalytic cytochrome enzyme in the human liver. This was partially attributed to the fact that CYP3A5 is highly polymorphic. However the importance of other underlying mechanisms remain unclear. The aim of the present study was to investigate the interaction between these enzymes. A human HepG2 hepatocellular line stably overexpressing CYP3A5 was constructed. The results suggested that CYP3A5 does not affect CYP3A4 expression directly. However, overexpression of CYP3A5 attenuated the inducibility of CYP3A4 in response to dexamethasone. A luciferase reporter assay indicated that this attenuation was due to a decrease in CYP3A4 promoter activity. Furthermore, a pharmacokinetic assay using quinidine and amlodipine showed that CYP3A4 enzyme activity per mg of microsomal protein was also decreased in the group overexpressing CYP3A5 compared with the dexamethasone-induced control group. In conclusion, the current study demonstrated that CYP3A5 may affect CYP3A4 at the transcriptional level and may thus modify CYP3A4 expression and activity in the presence of substrates and inducers. The results indicate that CYPs may interact with each other under certain conditions and that this interaction may be a novel mechanism by which drug-drug interactions are mediated.

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Introduction

The human cytochrome P450 (CYP) enzyme system is a superfamily of paralogs that are dominantly expressed in the adult liver and gastrointestinal tract (1). They catalyze phase-I metabolism of a wide variety of exogenous chemicals, resulting in substrates that are more water soluble, and thus facilitating excretion or further transformation into nontoxic compounds (2).

CYP3A4 is the predominant P450 enzyme expressed in human liver, and was understood to metabolize >60% of clinically prescribed drugs (3). CYP3A4 is not detectable prior to birth, but its expression gradually increases thereafter (4). Despite the heterogeneity of liver CYP3A4 expression among adult humans, CYP3A4 expression may also be affected transiently by xenobiotics, mostly its substrate, including CYP inducers, such as rifampin, phenobarbital, clotrimazole and dexamethasone (5), and inhibitors, such as verapamil, erythromycin, nifedipine, testosterone, midazolam and amiodarone (6). Changes in intestinal and liver CYP3A4 activity may have a significant effect on drug metabolism and thereby affect bioavailability of these compounds (7). Thus, increasing attention has been focused on the involvement of CYP3A4 in drug-drug interactions (DDI) (8,9).

The CYP3A5 gene is located on chromosome 7q22.1, upstream of CYP3A4, and shares 84% similarity in amino acid sequence with CYP3A4 (10). CYP3A4 and 5 catalyze similar and overlapping metabolic reactions, including nifedipine oxidation, testosterone 6β-hydroxylation, erythromycin N-demethylation, cyclosporine oxidation and hydroxylation of benzodiazepine, midazolam, triazolam, alprazolam and terfenadine (11). Although it may differ in catalytic activity and regioselectivity, some reports have argued that CYP3A5 is more important than CYP3A4 for overall drug clearance (12,13) and also in the pathogenesis of certain diseases, such as hypertension (14). It exhibited comparable or greater metabolic activity than CYP3A4 for certain substrates, including carbamazepine (12). Unfortunately, the majority of reported studies have not distinguished the activity of CYP3A4 from CYP3A5, and use CYP3A4 to reflect the activity of either enzyme. However, there are important differences between the enzymes. CYP3A5 is highly-expressed

in the adult kidney (15) rather than the liver, in contrast to CYP3A4. In addition expression of CYP3A5 in human liver varies among individuals and species from being undetectable to comprising >50% of total CYPs present in this organ (16). Therefore the importance of CYP3A5 in metabolism may have been underestimated.

A number of studies have emphasized the polymorphism of CYP3A5 and have investigated the relevance of this to clinical issues in treating hypertension (17), and during liver (18) and kidney (19,20) transplantation. However, conflicting results have been obtained. For example, a study examining the effect of CYP3A5 deactivating mutations, reported no association between expression of this enzyme and the response of blood pressure to amlodipine among African-Americans (21) By contrast, CYP3A5 expression had a significant impact on the response of blood pressure to amlodipine in healthy Korean subjects (22). In order to resolve this conflicting information, the present study investigated whether external factors are involved in modulating the expression of CYP3A5 in response to certain drugs. The effect of overexpression of CYP3A5 on the ability of dexamethasone (DEX) to induce CYP3A4 activity was investigated. The effect of CYP3A5 on the promoter activity of CYP3A4 in the presence of DEX was also examined. Finally the metabolism of a representative substrate drug, amlodipine in response to CYP3A5 overexpression was investigated.

Materials and methods

Chemicals and plasmid construction. Lipofectamine® 2000 (18324-012) was obtained from Invitrogen Life Technologies, (Carlsbad, CA, USA). The protein G-Sepharose (17-0618-01) was obtained from GE Healthcare Biosciences (Pittsburgh, PA, USA).. Monoclonal mouse anti-human CYP3A4 (sc-53850) and CYP3A5 antibodies (sc-53616) were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Monoclonal mouse anti-α-tubulin antibody (T5168), quinidine and amlodipine were purchased from Sigma-Aldrich (St Louis, MO, USA). Horseradish peroxidase-conjugated anti-mouse secondary antibody was obtained from GE Healthcare Biosciences. Other chemicals used to prepare buffers were obtained from Shenggong Ltd. (Shanghai, China). Dexamethasone was reconstituted in dimethyl sulfoxide (DMSO) at concentration of 1 mM.

The GFP-CYP3A5 plasmid was constructed by in-frame ligation of a CYP3A5 coding sequence amplified from HepG2 cDNA with pIRES2-eGFP (Clonetech Laboratories, Mountain View, CA, USA). pGL3-CYP3A4-promoter consisted of a pGL3-basic vector (Promega Corporation, Madison, WI, USA) inserted with the 5'-flanking region of CYP3A4 (from -1.6 kb to +100 bp to the start codon).

Cell culture and stable transfection of CYP3A5. Human HepG2 cells (Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China) were cultured from a nitrogen preserved batch and maintained with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in a humidified incubator at 37˚C with 5% CO_2. Cells ($5x10^5$) were seeded onto a 6-cm dish and transfected with 10 μg purified GFP-CYP3A5 plasmid at 70% confluence using Lipofectamine 2000 according to the manufacturer's instructions. Cells were then reseeded onto three 10-cm dishes with complete medium supplemented with 500 μg/ml of G418 (Cellgro, Manassas, VA, USA) for colony selection. Clones were selected 2 weeks post-transfection and expanded for RNA and protein verification. Positive CYP3A5$^+$ HepG2 clones were frozen for subsequent experiments.

Table I. Sequences of primers and siRNAs.

Primer and siRNA	Primer/siRNA sequences
CYP3A4 promoter	F: gttcacaggaagcagcacaaa
	R: gagagccatcactactttccttact
CYP3A5	F: attcagcaagaagaacaaggaca
overexpression	R: tggtgttctcaggcacagat
CYP3A4 RT	F: ccttacatatacacacccttggaag
	R: ggttgaagaagtcctcctaagct
CYP3A5 RT	F: aggcgggaagcagagaaag
	R: ggggtcttgtggattgttgag
CYP3A5 siRNA	Si-A: tgcctttgttgggaaatgttttg
	Si-B: tccattatttctctcaataatac
	Si-C: gagttattctaaggatttctact

siRNA, small interfering RNA; F, forward; R, reverse; RT, reverse transcription.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). HepG2 cells were seeded in 6-well plates at a density of $5x10^5$ cells per well Following indicated treatments, samples were washed with phosphate-buffered saline (PBS) and lysed with 1 ml TRIzol® (Invitrogen Life Technologies). RNA extractions were performed according to the manufacturer's instructions. Total RNA (1 μg) from each sample was used for cDNA synthesize using a Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). qPCR was then performed using All-in-One SYBR® Green qPCR mix (GeneCopoeia, Rockville, MD, USA) on an Applied Biosystem 7300 qPCR module. The sequences of CYP3A4/5 detecting primers are provided in Table I, and human GAPDH (23) was used as internal control. The qPCR conditions were set as follows: Pre-heating at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 15 sec, and a final melting curve stage. Fold changes in mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Protein and western blotting. Treated HepG2 cells were washed twice with PBS and lysed in radioimmunoprecipitation assay buffer (50 mm Tris, pH 7.5; 150 mm NaCl; 0.1% SDS, 0.5% sodium deoxycholate; 1% Triton X-100; and 1 mm phenylmethanesulfonyl fluoride). Cell lysates were then sonicated on ice for 30 sec and cell debris was removed by centrifugation at 10,000 x g for 15 min. The protein concentrations of each

sample were determined by a bicinchoninic acid assay (Pierce Biotechnology, Inc., Rockford, IL, USA) according to the manufacturer's instructions. Total protein (30 μg) was loaded onto 10% SDS-PAGE gels, and transferred to polyvinylidene fluoride membranes (GE Healthcare Biosciences). Each membrane was blocked with 5% non-fat milk and incubated with the indicated antibodies. The membranes were incubated with primary antibodies at room temperature for 4 h or at 4˚C overnight and horseradish peroxidase-conjugated anti-mouse secondary antibody (1:5,000) at room temperature for 1 h. Signals were visualized using Enhanced Chemiluminescence (ECL) western blotting substrate (Thermo Fisher Scientific). Monoclonal mouse anti-human CYP3A4/5 antibodies were diluted at a ratio of 1:1,000 in Tris-buffered saline containing 5% bovine serum albumin and 0.1% sodium azide. . Each blot was stripped using Stripping Buffer (0.5 mM NaCl/0.2 mM acetic acid) and reprobed. Monoclonal mouse anti-α-tubulin (1:3,000) was used as an internal control.

Luciferase reporter assay. Luciferase reporter assays were performed using a Dual-Luciferase® Reporter Assay system (Promega Corporation). Specifically, pGL3-CYP3A4-promotor Luciferase reporter vectors were co-transfected with or without CYP3A5 siRNA, as indicated, using Lipofectamine 2000 in 24-well plates, where HepG2 cells were at 70% confluence. Following treatment, cells were washed and lysed with 80 μl lysis buffer from the Dual-Luciferase®Reporter kit. Following three freeze and thaw cycles, cell lysates were centrifuged at 4˚C and 9,300 x g for 10 min and 10 μl was mixed with 100 μl of buffer LARII by pipetting in luminometer tubes. Firefly fluorescence was then read on a Turner DesignsTD-20/20 luminometer (Promega Corporation) immediately. The fluorescence of *Renilla* luciferase was measured in a second reading following the addition of 100 μl Stop & Glo®Reagent and vortexed for 5 sec at ~100 rpm.

High performance liquid chromatography-mass spectometry (HPLC-MS) detection of CYP3A4 substrate metabolites. Enzyme catalytic activity experiments were performed using microsomes extracted from treated HepG2 cells, as previously described (24). Microsomes were suspended in PBS (0.1 mM, pH 7.4). Microsomal protein (50 μg) in 250 μl total incubation volume was achieved in all samples following addition of substrates. Samples were pre-incubated for 5 min in a 37˚C shaker, and enzyme reactions were initiated by applying nicotinamide adenine dinucleotide phosphate (NADPH) regenerating buffer to a working concentration of 1 mM $NADP^+$, 7.5 mM isocitric acid, 10 mM magnesium chloride and 0.2 units of isocitric dehydrogenase. Total organic solvent did not exceed 1% v/v. The linear range of each substrate was determined. Quinidine was chosen as a selective probe for CYP3A4 and amlodipine, widely used clinically as an antihypertensive, was used as a representative CYP3A4-metabolized drug. A starting concentration of 1 mM quinidine and amlodipine was used for the 20 min incubation. Reactions were terminated with 100 μl acetonitrile containing 0.1 μM tolbutamide as an internal standard. Samples were then analyzed by HPLC- MS, as previously described (25).

Statistical analysis. The data were collected by at least three independent experiments. Averages and standard deviations were calculated using GraphPad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software. Student's t test was used for evaluating differences and a $P<0.05$ was considered to indicate a statistically significant difference.

Results

Overexpression of CYP3A5 does not affect the expression of CYP3A4. Increasing evidence has demonstrated that CYP3A5 is important in the pathogenesis of hypertension (14), not only via the control of water retention (26,27), but also through the metabolism of certain antihypertensive drugs. However, conflicting data have been reported regarding the influence of CYP3A5 on the response of blood pressure to amlodipine (21,28). It was hypothesized that there may be additional factors involved in moderating the metabolism of drugs other than the innate catalytic ability of CYP3A5.

The effect of CYP3A5 on CYP3A4 expression was investigated in a HepG2 cell line, which was induced to overexpress CYP3A5 using GFP-CYP3A5. The expression level of CYP3A5 was confirmed by western blotting and RT-qPCR. As shown in Fig. 1, RNA and protein levels of CYP3A4 were not affected by CYP3A5 overexpression, suggesting that CYP3A4 is not direct regulated by CYP3A5.

Overexpression of CYP3A5 reduces induction of CYP3A4 expression by DEX. The effect of clinical conditions, such as the presence of substrates or inducers was then investigated. DEX was selected due to its wide range of clinical applications. HepG2-wild type (WT) and HepG2-$CYP3A5^+$ cells were seeded onto 6-well plates at $5x10^5$ cells/well. Following overnight adhesion, 100 nM DEX was applied for a further 48 h incubation. Cells were then harvested for RNA and protein analysis. As hypothesized, CYP3A4 RNA as well as protein levels were significantly elevated following DEX induction in the WT groups compared with the control group (Fig. 2). However, this induction was suppressed in the cells overexpressing CYP3A5. This indicates that CYP3A5 affects CYP3A4 indirectly under certain conditions.

Overexpression of CYP3A5 attenuates CYP3A4 promoter activity in the presence of DEX. To further explore the effect of overexpression of CYP3A5 on DEX induction of CYP3A4 expression, the 5'-flanking region of CYP3A4 was cloned as described. Three short interfering RNAs (siRNAs) were designed and synthesized at GenePharma Ltd. (Shanghai, China), and transfected into HepG2 cells. The effects of silencing CYP3A5 were examined with western blotting and the most potent siRNA (Si-C) was selected for subsequent experiments (Fig. 3). HepG2-WT and HepG2-$CYP3A5^+$ cells were seeded onto 24-well plates at a density of $4x10^4$ cells/well. The purified pGL3-CYP3A4-promoter plasmid was co-transfected with Si-C or control RNA following cell adhesion. Fresh medium containing 100 nM DEX was exchanged at 6 h post-transfection. Samples were harvested, and Luciferase activity was measured, following 48 h incubation as described. In concordance with the CYP3A4 expression data shown above, the CYP3A4 promoter activity was unaffected by

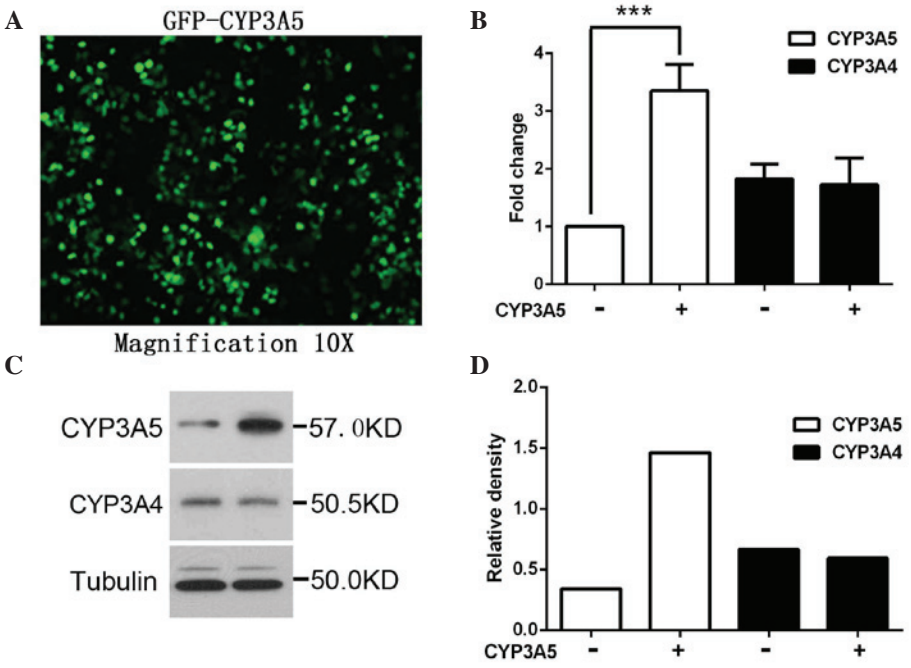

Figure 1. Effect of overexpression of CYP3A5 on the expression of CYP3A4. (A) A HepG2 cell strain stably overexpressing CYP3A5 was constructed. (B) mRNA levels of CYP3A4/5 of WT and CYP3A5$^+$ HepG2 cells were measured using reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean ± standard deviation (n=3). ***P<0.001. (C) Protein analysis of CYP3A4/5 of WT and CYP3A5$^+$ HepG2 cells using western blotting. (D) Density quantitation of western blot signal shown in panel C. CYP, cytochrome P450 (subsequent characters denote family, subfamily and polypeptide); WT, wild type.

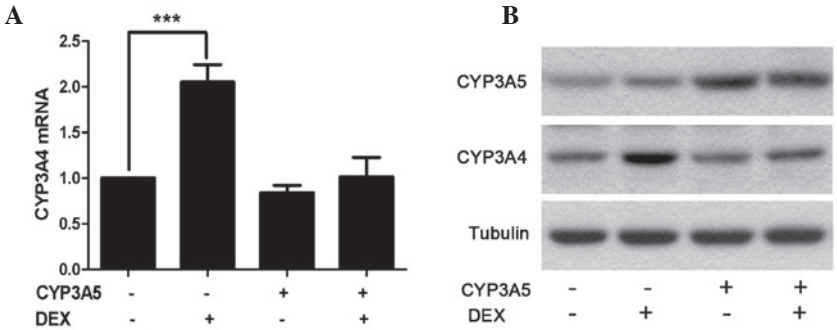

Figure 2. Effect of overexpression of CYP3A5 on DEX-induced CYP3A4 expression. (A) Reverse transcription-quantitative polymerase chain reaction detection of CYP3A4 mRNA levels in WT or CYP3A5$^+$ HepG2 cells treated with or without DEX. Data are presented as the mean ± standard deviation (n=3). ***P<0.001. (B) Protein expression of CYP3A4/5 following DEX application on WT or CYP3A5$^+$ HepG2 cells. CYP, cytochrome P450 enzyme (subsequent characters denote family, subfamily and polypeptide); DEX, dexamethasone; WT, wild type.

overexpressing or silencing of CYP3A5. DEX was shown to stimulate activation of the CYP3A4 promoter, which was also consistent with previous reports. Notably, overexpression of CYP3A5 suppressed CYP3A4 promoter activity compared with control in the presence of DEX. This suggests that an abundance of CYP3A5 may affect CYP3A4 expression at the transcriptional level under certain conditions.

Overexpression of CYP3A5 decreases the activity of CYP3A4 in the presence of DEX. To further investigate the impact of the suppression of CYP3A4 function in the presence of DEX, CYP3A4 activity in cells that had undergone the same treatment was measured. In order to remove the influence of CYP3A5, quinidine was used as a selective CYP3A4 probe based on the availability of clinical DDI data and the structural characteristics of the probe substrate (20). The 3-hydroxylated quinidine generated in each group was normalized to non-treated controls, in order to assess the alteration in CYP3A4 activity. As in the luciferase experiments, the enzyme activity was also reduced compared with DEX-treated controls (Fig. 4A). Greater activity was observed in the CYP3A5-silenced group. CYP3A4 levels appeared to be inversely associated with the expression of CYP3A5 in the presence of DEX.

The impact of this interaction on potential clinical events was examined using amlodipine, a calcium channel blocker, widely used as an antihypertensive drug. The un-transformed amlodipine was measured by HPLC-MS/MS and normalized to the levels in the DEX-untreated control (Fig. 4B). The rate of amlodipine metabolism increased in cells overexpressing CYP3A5, although it was not statistically significant. The

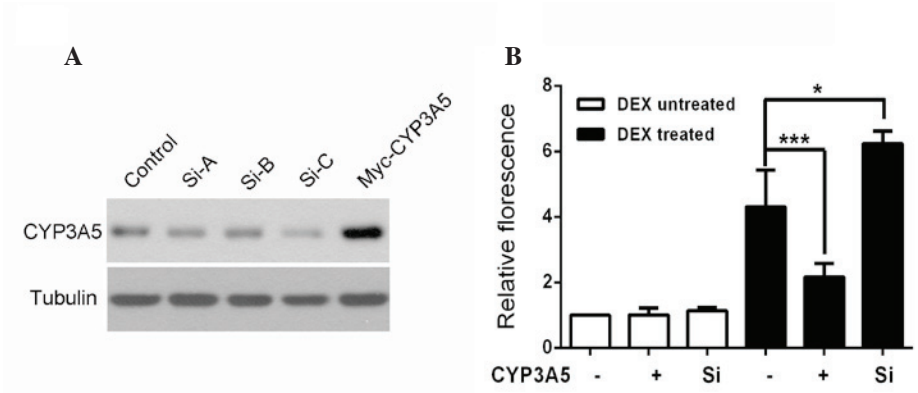

Figure 3. Effect of overexpression of CYP3A5 on CYP3A4 promoter activity in the presence of DEX. (A) Potency of CYP3A5 siRNAs were evaluated by WB. Myc-CYP3A5 was used as transfection control. (B) Promoter activity of CYP3A4 was detected by a luciferase activity assay. -, WT HepG2 cells; +, CYP3A5^{+}-overexpressing cells; Si, CYP3A5 silenced cells. Data are presented as the mean ± standard deviation (n=3). *P<0.05 and ***P<0.001. CYP, cytochrome P450 enzyme (subsequent characters denote family, subfamily and polypeptide); DEX, dexamethasone; siRNA, small interfering RNA; WT, wild type.

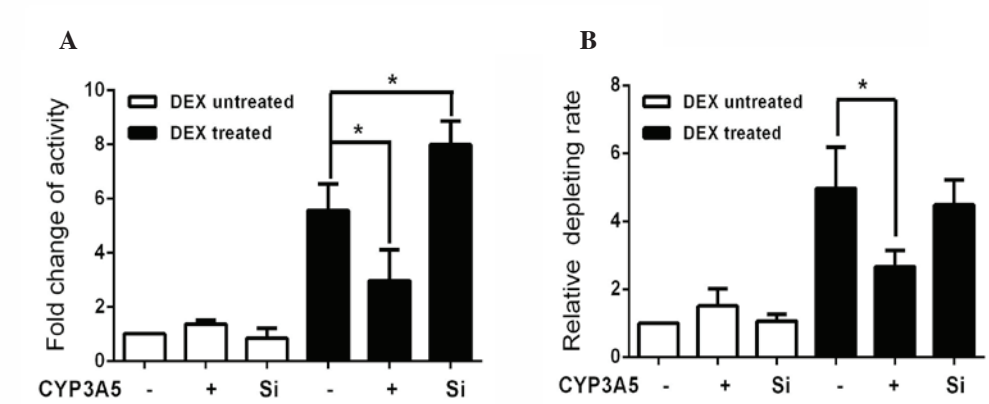

Figure 4. Effect of overexpression of CYP3A5 on CYP3A4 enzyme activity following DEX application. (A) CYP3A4 activity of HepG2 microsome detected by quinidine. (B) Amlodipine metabolic dynamics. All data were normalized to the first column. -, WT HepG2 cells; +, CYP3A5^{+}-overexpressing cells; Si, CYP3A5 silenced cells. Data are presented as the mean ± standard deviation (n=5). *P<0.05. CYP, cytrochrome P450 enzyme (subsequent characters denote family, subfamily and polypeptide); DEX, dexamethasone; WT, wild type.

clearance of amlodipine increased following DEX induction, and this induction was reduced by CYP3A5 overexpression. This may be attributed to the fact that the metabolism of amlodipine by CYP3A4 is four times higher than that of CYP3A5. Following DEX induction, the rate of metabolism of amlodipine in the CYP3A5-silenced group was not significantly changed compared with the DEX-treated control group. This result indicates that CYP3A4 is the primary enzyme involved in the metabolism of amlodipine. However, CYP3A5 may influence CYP3A4 metabolism of this and other substrates following exposure to DEX.

Discussion

Primary hepatocytes are a valuable *in vitro* model with which to identify compounds that are potentially toxic to humans. However, there are a number of disadvantages to the use of primary hepatocytes, including a shortage of available human liver material, limited proliferation ability and loss of metabolic activity after a limited number of passages, which have constrained its application. A number of groups have explored the possibility of obtaining hepatocytes from differentiated adult or embryonic stem cells, or immortalized human hepatocytes, but this technique remains inconvenient. HepG2 cells express the predominant liver functional CYP isoforms associated with drug metabolism. CYP3A7 is the dominant isoform in these cells compared with CYP3A4 in the human adult liver. Thus, HepG2 cells possess a phenotype most similar to that of the fetal liver (29,30). Although less suitable for predicting effects on metabolism in humans, HepG2 cells provide a system that is easy to handle and reproducible, and thus suitable for the investigation of gene regulation. Quinidine was reported to be predominantly metabolized by CYP3A4 (25), which is consistent with the observation in the present study that overexpression of CYP3A5 did not increase the rate of quinidine metabolism significantly in the absence of other compounds. The results confirm that quinidine is a reliable marker of CYP3A4 activity.

Environmental factors, such as cigarette smoking and food intake induce the expression of CYPs and increase clearance of phenacetin and theophylline (31). Hepatic diseases, such as hepatitis B virus infection and cirrhosis, and age, gender, hormones, inflammation and pregnancy alter the expression pattern of CYP enzymes (32). The complexity of the transcriptional regulation of CYP3A4 has been attributed to the response of CYP3A4 to such factors. Transcription factors, such as

pregnane X receptor (PXR; -362/+53) (33), constitutive androstane receptor (CAR) (34), nuclear factor I (-243/-220) (29), differentially expressed in chondrocyte 1 (35) and hepatocyte nuclear factor 4a (HNF4a) (36) have been reported to account for a component of CYP3A4 inter-individual variability. Constitutive liver enhancer module of CYP3A4 (CLEM4) (37) and CCAAT-enhancer-binding proteins (C/EBP) response elements (38) were also found within its proximal promoter. However a significant degree of CYP3A4 variation remains unexplained. Epigenetic regulation of CYP3A4 has also been explored recently. The 12 kb CYP3A4 regulatory region shows highly variable CpG methylation in the adult liver, which corresponds to important CYP3A4 transcription factor binding sites, including xenobiotic responsive enhancer module, CLEM4, C/EBP and HNF4a (39). In addition, a high degree of methylation was observed in the fetal liver, which is consistent with the minimal expression of CYP3A4 at this stage. The results from the promoter activity experiments demonstrated that CYP3A5 does not affect CYP3A4 transcription directly. However, in the presence of DEX, the promoter activity appears to be inversely correlated with the expression of CYP3A5 (Fig. 3). This data may indicate that excessive CYP3A5 prevents DEX from binding to its response elements. However, which transcriptional factors are involved remains to be elucidated.

CYP3A5 protein expression was found to be highly variable in a manner that was generally independent of age but dependent on race (4). The expression of the CYP3A5*3 polymorphism results in a truncated mRNA (40). This polymorphism is observed at a similar frequency in Chinese and Japanese populations, but three times higher in Caucasian populations (41,42), which implies that more Asian subjects are extensive CYP3A5 metabolizers. CYP3A5 expression appears to be inducible via the glucocorticoid receptor, PXR and CAR-β, as is the case for CYP3A4. However, the 5'-flanking regions of CYP3A5 shares only 60% sequence similarity with that of CYP3A4. The low homology may be one of the factors that differentiates their regulation (43). This may explain the different effects of DEX on the induction of the expression of each of these genes (Fig. 2). CYP3A5 has been associated with disease, however these associations are independent to its drug metabolizing function. CYP3A5*1 homozygotes may have higher systolic blood pressure (14). Certain combined CYP3A4/CYP3A5 haplotypes exhibit differential susceptibility to prostate cancer (44). Females positive for CYP3A5*1 appear to reach puberty earlier, which may affect their risk of developing breast cancer (45). The results from the current study demonstrating an interaction between CYP3A5 and CYP3A4 extends the potential impact of CYP3A5 polymorphisms and variations in expression, since the metabolic capacity of CYP3A4 appears to be higher than of CYP3A5 and CYP3A7 (46) for the majority of substrates.

DEX is a potent synthetic member of the glucocorticoid class of steroid drugs that are widely used as anti-inflammatory and immunosuppressant treatments. DEX is preferentially metabolized by CYP3A4 into 6β-OH-DEX in the human adult liver. Thus DEX has been used in a number of studies as a probe for CYP3A4 activity. However, DEX is also a potent inducer of CYP3A4 (47). Evidence reported by Pascussi *et al* (48) demonstrated that the mechanism underlying DEX induction of CYP3A4 is concentration-related, as a low dose (10-100 nM) of DEX induced CYP3A4 via the glucocorticoid receptor, whilst a high concentration (10 μM) activates CYP3A4 through the PXR pathway. In the present study, DEX strongly induced CYP3A4 in HepG2 cells, whilst the induction by CYP3A5 was limited (Fig. 2). This is consistent with previous reports (30,46). Since DEX is also a common substrate of CYP3A5, although at a relatively lower metabolic rate, it is postulated that the overexpression of CYP3A5 accelerates the metabolism of DEX, and thus reduces the level of expression of CYP3A4 that it can induce. Thus, DEX may be a bridge linking CYP3A4 and CYP3A5 function. A similar observation was made that DEX increased erythromycin breath test (ERBT) only in CYP3A5*1 non-carriers as they may be more susceptible to the inductive effects of DEX due to lower basal CYP3A activity (49).

Adverse drug interactions are an important cause of morbidity, hospitalization, and mortality. Drug interactions are be the fourth leading cause of death in hospitalized patients in the US (50). The greatest risk of drug interactions occurs due to effects on the cytochrome system. CYP3A4, the most prevalent cytochrome P450, accounts for 30-50% of drugs metabolized by type I enzymes. Previous DDI studies have focused on drug metabolism by single specific enzymes, such as CYP3A4 or CYP3A5. However, conflicting results have been reported using this model. For instance, CYP3A5*3 carriers require a lower dose of substrate drugs, such as cyclosporine and tacrolimus (51). However, CYP3A5*3 showed no association with the response of blood pressure to amlodipine in African-Americans with early hypertensive renal disease (21). The current study demonstrated that the contribution of CYP3A5 may be an important source of inter-individual variability in response to drugs. Furthermore, the identification of this novel interaction may provide further insights when predicting drug metabolism and designing individualized treatment regimes, particularly when a patient with multiple co-morbidities is prescribed more than two drugs.

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