

The role of CYP3A4 in the biotransformation of bile acids and therapeutic implication for cholestasis

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Abstract: CYP3A4 is a major cytochrome P450. It catalyses a broad range of substrates including xenobiotics such as clinically used drugs and endogenous compounds bile acids. Its function to detoxify bile acids could be used for treating cholestasis, which is a condition characterised by accumulation of bile acids. Although bile acids have important physiological functions, they are very toxic when their concentrations are excessively high. The accumulated bile acids in cholestasis can cause liver and other tissue injuries. Thus, control of the concentrations of bile acids is critical for treatment of cholestasis. CYP3A4 is responsively upregulated in cholestasis mediated by the nuclear receptors farnesol X receptor (FXR) and pregnane X receptor (PXR) as a defence mechanism. However, the regulation of CYP3A4 is complicated by estrogen, which is increased in cholestasis and down regulates CYP3A4 expression. The activity of CYP3A4 is also inhibited by accumulated bile acids due to their property of detergent effect. In some cholestasis cases, genetic polymorphisms of the CYP3A4 and PXR genes may interfere with the adaptive response. Further stimulation of CYP3A4 activity in cholestasis could be an effective approach for treatment of the disease. In this review, we summarise recent progress about the roles of CYP3A4 in the metabolism of bile acids, its regulation and possible implication in the treatment of cholestasis.

Keywords: CYP3A4; cholestasis; nuclear receptors; bile acids; detoxification



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Introduction

Cytochrome P450s (CYPs) are a superfamily of enzymes, which catalyse a broad range of compounds (1,2). These enzymes detoxify xenobiotics to exert body defence against environmental hazard. They also play key roles in carcinogenesis by turning precarcinogens into carcinogen such as azoxymethane and deactivating carcinogens (3). CYPs also regulate many human body biological processes such as biosynthesis of estrogen, conversion of cholesterol into bile acids and biotransformation of bile acids (4). Manipulation of these processes through CYP activities may help prevention and treatment of diseases. In this review, we summarise the roles of CYP3A4 in biotransformation of bile acids, regulation of CYP3A4 and stimulation of CYP3A4 activity for the treatment of cholestasis.

Bile acids are the major products of cholesterol catabolism, which have important physiological functions in human body. They facilitate not only the elimination of cholesterol and body wastes but also the digestion of fat and the absorption of lipid soluble vitamins. Bile acids also play a role as signalling molecules in the fine-tuning of energy homeostasis (5,6). However, bile acids are toxic when accumulated in high concentration. Cholestasis is the impairment of bile flow and is characterised by the accumulation of bile acids in the liver and serum (7). The accumulated bile acids are toxic and critical in the pathophysiology of cholestasis. A major aim of cholestasis treatment is to reduce the concentration of toxic bile acids in both blood and liver.

The toxicity of accumulated bile acids has been

Table 1 Substrates, inducers and inhibitors of CYP3A4

Types	Examples of agents (Ref)
Substrates	Alfentanil (14), Amiodarone (13), azole antifungal (14), Amprenavir (14), Phenobarbital (14), Benzodiazepine (14), Carbamazepine (15), Codeine (14), Cyclosporine (14), Cyclophosphamide (16), Erythromycin (14), Estradiol (14), Fexofenadine (14), HMG-CoA reductase inhibitors (14), Hydrocortisone (14), Imatinib (14), Lidocaine (14), Midazolam (17), Nifedipine (17), Quinidine (14) Ritonavir (14), Sirolimus (14), Tacrolimus (14) Tamoxifen (14) Terfenidine (14) Testosterone (17)
Inducers	Phenobarbital (14), Carbamazepine (15), Dexamethasone (18), Hyperfoin (19), Efavirenz (20), Nevirapine (20), Phenytoin (18), Rifampicin (14), Bile acids (19)
Inhibitors	Amiodarone (13), Azole antifungals (17), Bergamottin (21), Bile acids (9), Pomegranate juice (15) Cyclosporin (22), Gestodene (22), Macrolide antibiotics (22), Midazolam (22), Non-nucleoside reverse transcriptase inhibitors (22), Protease inhibitors (17), Raloxifene (22), Tamoxifen (22), Verapamil (22)

shown both *in vivo* and *in vitro*, which result in a series of pathological changes including alterations of P450 activity. In a 5 day bile duct ligated model, total P450 was decreased to 50% and 75% of controls in male and female rats respectively (7,8). *In vitro*, addition of bile acids dramatically inhibited P450-catalysed reactions (9). The effect correlates with the hydrophobicity of bile acids with most toxic being the lithocholic acids, the moderately toxic deoxycholic and chenodeoxycholic acids and the least toxic cholic acid. At a cellular level, bile acids caused apoptosis at low concentrations and necrosis at high concentrations.

There are adaptive responses in the body to decrease the pool of bile acids to protect the body from their toxicity. These responses include decreased synthesis, increased metabolism and renal elimination of bile acids. Nuclear receptors have the important functional roles in these responses and hold much promise in the treatment of cholestasis (10). CYP3A4 is the major enzyme for the detoxification of bile acids, hydrolysing bile acids to facilitate their subsequent glucuronidation by UDP-glucuronosyltransferases (UGTs) (11). Stimulation of CYP3A4 activity could be employed for the treatment of cholestasis.

Role of CYP3A4 and its regulation

CYP3A4 is a member of cytochrome P450 (CYP450) enzymes encoded by a gene superfamily (12). Among all CYP enzymes, CYP3A4 is the most important, which is the most abundant in both liver and intestinal tract (13). The gene encoding CYP3A4 is located in chromosome 7q21.1. Other CYP enzymes encoded in 7q21.1 are CYP3A5, CYP3A7 and CYP3A43. But they are minor isoforms.

The enzyme CYP3A4 has broad substrates (Table 1).

The broad substrates are attributed to the structure of the CYP3A4 active site which has been characterised by X-ray crystallography (23). It has been reported that CYP3A4 has a much larger active site cavity to bind a broad range of substrates, compared with other CYPs (23,24). CYP3A4 also has multiple binding sites (25). The substrates are generally transformed into inactive forms or compounds of lower activity through reactions such as N-oxidation, C-oxidation, N-dealkylation, O-dealkylation, nitro-reduction, dehydration and C-hydroxylation. CYP3A4 metabolises not only xenobiotics including majority of drugs and carcinogens to protect living organisms from environmental toxicity (12,26) but also many endogenous compounds such as cholesterol, bile acids, fatty acids, prostaglandins, leukotrienes, retinoids and biogenic amines (12,27).

CYP3A4 expression, its stability and inhibition are regulated by different mechanisms. Many factors including nuclear receptors, hormones, xenobiotics and signalling molecules regulate CYP3A4 expression as shown in Figure 1 (27,28). In CYP3A4 regulatory region, there are two binding sites for nuclear receptors PXR and FXR to bind to and confer transactivation (29). PXR forms a heterodimer with 9-cis retinoic acid receptor (RXR) to bind to CYP3A4 promoters (14,30,31). Disruption of these elements in the promoter of CYP3A4 caused dramatic decrease in reporter activities (30). CYP3A4 dis-regulation in PXR-null mice further demonstrated an important role of PXR in CYP3A4 activation (32). Another nuclear receptor constitutive androstane receptor (CAR) has also been demonstrated to activate CYP3A4 (33,34). Many hormones such as triiodothyronine, dexamethasone and growth hormone involve the regulation of CYP3A4 (20). CYP3A4 is also regulated by a large number of xenobiotics

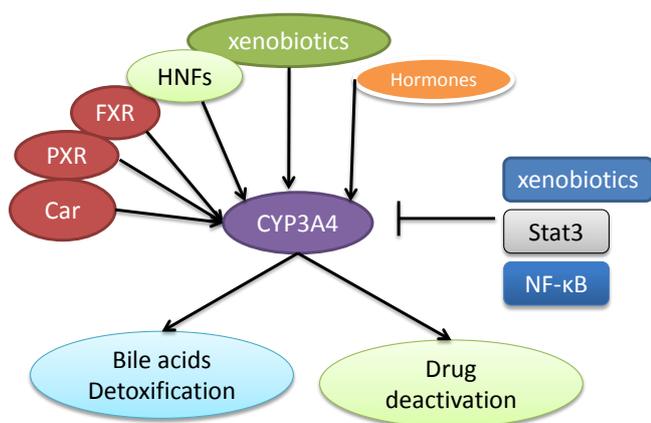


Figure 1 Regulation of CYP3A4. CYP3A4 is stimulated by nuclear receptors (PXR, FXR, CAR), hormones, xenobiotics and HNFs. It is inhibited by xenobiotics and signalling molecules Stat3 and NF- κ B. Increased CYP3A4 activity results in increased bile acids detoxification and drug deactivation. Abbreviations: FXR, farnesol X receptor; PXR, pregnane X receptor; CAR, constitutive androstane receptor; HNF, Hepatic nuclear factor; Stat3, signal transducer and activator of transcription 3 and NF- κ B nuclear factor kappa beta.

including many drugs (*Table 1*) (18,21,31,35). Several drugs, some beverage and bile acids can inhibit CYP3A4 activity (9,15,22,36-38).

Several signalling molecules have also been demonstrated to regulate CYP3A4. These molecules include C/EBP α and C/EBP β , HNF1 α and HNF3 γ , HNF4 α , USE, AMPK and PPAR- γ (39-43). Jover *et al.* showed that JAK/Stat pathway down-regulates CYP3A4 (39). NF- κ B was also shown to decrease CYP3A stability (44).

CYP3A4 in bile acids transformation and its alterations in cholestasis

CYP3A4 also plays an important role in the detoxification of bile acids where it catalyses their hydroxylation thereby increasing the hydrophilicity of bile acids and thus decreasing their toxicity. Bile acids are synthesised from cholesterol and rate-limiting enzyme is CYP7A1. It is subject to feedback inhibition by bile acids. The bile acids are also metabolised by CYP3A4 (45,46), which exerts an essential protective effect in cholestasis. Several studies have characterised the products formed by CYP3A4 from different bile acids (*Figure 2*). Chenodeoxycholic acid is turned into both hyocholic acid (3 α , 6 α , 7 α -trihydroxy-5 β -cholanoic acid) and 3 α , 7 α -dihydroxy-3-oxo-5 β -cholanoic acid while

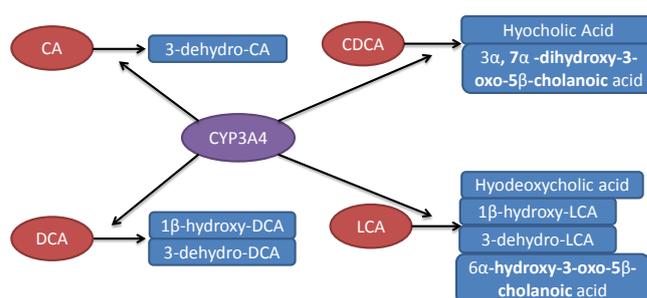


Figure 2 Biotransformation of bile acids by CYP3A4. CYP3A4 transforms CA into 3-dehydro-CA, CDCA into Hyocholic Acid and 3 α , 7 α -dihydroxy-3-oxo-5 β -cholanoic acid, DCA into 1 β -hydroxy-DCA and 3-dehydro-DCA and LCA into hyodeoxycholic acid, 1 β -hydroxy-LCA, 3-dehydro-LCA and 6 α -hydroxy-3-oxo-5 β -cholanoic acid. Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

only one product is formed from cholic acid, 3-dehydro-CA (7 α , 12 α -dihydroxy-3-oxo-5 β -cholanoic acid) (47,48). Deoxycholic acid (DCA) is catalysed into 3-dehydro-DCA (12 α -hydroxy-3-oxo-5 β -cholanoic acid) and 1 β , 3 α , 12 α -trihydroxy-5 β -cholanoic acid (47,48). Lithocholic acid was metabolised into four products 3-oxo-5 β -cholanoic acid (3-dehydro-LCA), 6 α -hydroxy-3-oxo-5 β -cholanoic acid, 3 α , 6 α -dihydroxy-5 β -cholanoic acid (hyodeoxycholic acid) and 1 β , 3 α -dihydroxy-5 β -cholanoic acid (1 β -hydroxy-LCA) (47,49,50). Patients treated with the antiepileptic drug carbamazepine, a CYP3A4 inducer, markedly elevated urinary excretion of 1 β -hydroxydeoxycholic acid. Both taurochenodeoxycholic acid and lithocholic acid have been subjected to metabolism by fourteen recombinant expressed CYPs (51). Actually, only CYP3A4 was able to catalyse 6- α -hydroxylation of these two bile acids. The V $_{max}$ and K $_{m}$ for 6- α -hydroxylation of taurochenodeoxycholic acid catalysed by CYP3A4 was 18.2 nmol/nmol P450/min and 90 μ M, respectively. Furthermore, 6- α -hydroxylation of these two bile acids has been found to correlate with the levels of CYP3A4 and 6- β -hydroxylation of testosterone in ten human liver microsomes (51). These reactions are completely inhibited by 10 μ M troleandomycin, a specific inhibitor of CYP3A4 (51).

It has been shown that CYP3A4 is distinctly increased in several cholestasis models and also upregulated in bile duct-ligated rats (BDL) (52). This could facilitate detoxification of bile acids and increase their renal elimination. However, increased bile acids have detergent effect, which decreases

the CYP3A4 activity (9,53).

Changed bile acid pool size by CYP3A4

Both liver and kidney are the two organs involved in the excretion of toxic substrates with each demonstrating complementary effects. In cholestasis, the major route for bile acid secretion through liver is blocked and thus, excretion by the kidney is greatly increased. In cholestasis patients, there was increased urinary excretion of 6 α -hydroxylated bile acids, α -hyocholic acid and hyodeoxycholic acid (54-56). In cholestasis of pregnancy, there was a ten- to one hundred-fold increase in cholic and chenodeoxycholic acid in the urine while the amounts of 1- and 6-hydroxylated products of cholic acid were also markedly increased (57).

In bile duct ligated rats, urinary excretion of bile acids was increased by more than 380-fold from 0.05 to 19 μ mol/day (58). The urinary excretion of 6 β -hydroxylated bile acids and β -muricholic acid also increased (16,58,59). The ratio of cholic acid/ β -muricholic acid in urine changed from 5:3 at day 1 to 1:8 at day 5 (16). This indicates metabolism of bile acids by CYP3A4 is increased in cholestasis and bile acids are detoxified and easier to be excluded through urinary.

Up-regulation of CYP3A4 expression in cholestasis

The induction of CYP3A4 expression by bile acids is mediated by FXR (30,60) and PXR (52,60,61). It has been reported that chenodeoxycholic acid enhance CYP3A4 expression via FXR (29). The regulatory sequences have recently been identified (62). There are two functional FXR recognition sites located in a 345 bp element within the regulatory region of CYP3A4 (29).

PXR forms a heterodimer with retinoid X receptor (RXR) to bind to xenobiotic response elements in the CYP3A4 promoter (63-66). Disruption of the PXR gene eliminated the enhanced expression of CYP3A4 by its inducers dexamethasone and pregnenolone 16 α -carbonitrile (32). In PXR-null mice, introduction of human PXR allowed the induction of CYP3A4 by the human-specific inducer-rifampicin (32). In the regulatory region of CYP3A4 gene, two PXR elements (distant and proximal) have been identified that are separated by more than 7,000 nucleotides in the genome (30). Disruption of the distant and proximal elements causes a 73% and 53% decrease in

reporter activities respectively. However, *in vitro* binding studies showed that the proximal element was preferred. Chromatin immunoprecipitation experiments showed that the genomic fragment harbouring the proximal element was preferably precipitated over the fragment containing the distant element in the CYP3A4 gene (67).

Bile acids were demonstrated to regulate PXR in an *in vitro* assay employing a chimeric reporter system in which the FXR ligand-binding domain was fused with a reporter gene. Addition of lithocholic acid, deoxycholic acid and chenodeoxycholic acid activated the reporter construct 5 fold (68). This was confirmed by the treatment of mice with lithocholic acid, 4 days of lithocholic acid administration caused liver necrosis in 50% of wild mice increasing to 100% in PXR-deficient mice (32). Another study demonstrated that PXR was upregulated by lithocholic acid and its 3-keto metabolite (19).

Other nuclear receptors such as CAR may be also involved in the regulation of CYP3A4 (69). CAR has been demonstrated to regulate CYP3A4 transcription (30). It has a crosstalk with PXR to regulate overlapping but distinct genes. However, so far, no evidence supports the binding of bile acids to CAR.

Bile acids have been also shown to bind to the vitamin D receptor with low affinity to activate CYP3A4 (70-73). Lithocholic acid was shown to activate CYP3A4 mainly via the vitamin D receptor in colon, where PXR appeared not involved as Dex failed to stimulate CYP3A4 expression. Other bile acids appear not to bind to this receptor. However, a lithocholic acid derivative—lithocholic acid acetate was 30 times more efficient at activating CYP3A4 than lithocholic acid (72). The binding model of lithocholic acid is different to that of 1,25-dihydroxyvitamin D₃ which has a higher receptor affinity. Thus, two vitamin D receptor elements with distinctly different DNA motifs were identified in the regulatory region of CYP3A4. This indicates that these two compounds have the complementary roles in CYP3A4 mediated detoxification. However, it remains unclear whether the vitamin D receptor plays a role in the expression of CYP3A4 by bile acids in the liver.

Detergent effect of bile acids on CYP3A4 activity and regulatory role of estrogen

Accumulated bile acids can inhibit CYP3A4 activity. As shown *in vitro*, they have non-specific detergent effects on several rat CYPs and non-CYPs (9). It is quite possible that CYP3A4 is also subjected to the direct inhibition of

accumulated bile acids. This should be taken into account when considering the actual activity of CYP3A4 in cholestasis.

Estrogen has an important role in cholestasis. It could cause cholestasis especially in patients with bile acid transporter deficiency. It has been found to accumulate in BDL rats (7,8) and to down-regulate male specific CYPs 2C11 and 3A2. Recently, estrogen was considered to down-regulate CYP3A4 in human liver. After comparing 27 human hepatic CYP3A4 mRNA levels, it was found that young females have lower levels of CYP3A4 mRNA than young men, while older women displayed the same levels of CYP3A4 as older men, both of which were much higher than those in young women (74). Thus, the implication is that estrogen may play a role in regulating CYP3A4 transcription. However, another study using 54 samples of human liver microsomes did not find any influence of age or gender on CYP3A4-mediated midazolam hydroxylation and CYP3A4 protein mass (75).

Overall, activity of CYP3A4 will be determined by (I) transcriptional up-regulation of CYP3A4 expression by bile acids through nuclear receptors, (II) down-regulation by blood levels of estrogen and (III) direct inhibition of CYP3A4 by bile acids due to detergent effect of bile acids. Therefore, CYP3A4 activity may be stimulated at the early stage of cholestasis but inhibited when cholestasis further develops due to highly increased blood levels of estrogen and bile acids.

Polymorphism in CYP3A4 mediated bile acids detoxification

CYP3A4 expression is highly variable. There are marked individual variations in the expression of CYPs including CYP3A4, which affect the biological response (efficacy and/or toxicity) of many drugs as well as individual responses to CYP inducers (76,77). In humans, a 10-fold variation exists among individuals for rifampicin induced CYP3A4 expression (78-80). In the liver, substantial differences in CYP3A4 expression between individuals have been detected that could not be explained by environmental factors (78). A similar situation was found in the intestine, whereby CYP3A4 mRNA levels in biopsies of the mucosa of five healthy subjects varied by about 10 fold (79). This variation could be related to the polymorphisms of CYP3A4 and the other genes involved in the regulation of CYP3A4.

Any polymorphism in the genes involved in CYP3A4 mediated bile acid detoxification in cholestasis could affect

this adaptive response. More than 20 CYP3A4 mutations have been identified in humans although the frequency is low (81). Mutations of CYP3A4 affect the metabolism of clinically used drugs, leading to toxic blood concentration of drugs. Theoretically, these CYP3A4 variations can also affect bile acid detoxification. However, there have been no reports on them as there have been for bile acids transporters. There is also a report that a high degree of SNP polymorphism in PXR found in a screening of the Dutch population (66). How these mutations affect the cholestasis is not yet assessed.

Therapeutic implications

The nuclear receptor activators have been extensively studied and some of them are actually used clinically. Rifampicin, for example, is used for treatment of cholestasis (82-86). In 14 healthy volunteers, administration of rifampicin at 600 mg for 7 days markedly increased urinary excretion of bile acids, the 6- α -glucuronides, caused by increased production of 6 hydroxylated hyocholic and hyodeoxycholic acids, with subsequently conjugation at C-6 by UDP-glucuronosyltransferase (87).

Ursodeoxycholic acid (UDCA) is used extensively although SW4064 and 6ECDCA have been shown to be much more potent inducers (86). UDCA has been used clinically for many years in the treatment of cholestasis. Although it was shown to have beneficial effect on the liver with cholestasis, the effect was mild (10). More effective compounds could be tested to improve the treatment of cholestasis.

As shown in *Figure 1* and *Table 1*, CYP3A4 activity is stimulated by many other compounds. These CYP3A4 activators may also be considered for use in treating cholestasis. There are possibilities to discover a better compound than UDCA and rifampicin in terms of effectiveness in reducing the levels of bile acids both in liver and blood. It is also possible to use some of them for combinatorial application with rifampicin or UDCA. At present these stimulators are not well studied in the treatment of cholestasis.

CYP3A4 catalyzes more than 50% of clinically used drugs. Any change of CYP3A4 activity will affect the pharmacokinetics of these drugs. Thus, the manipulation of CYP3A4 in cholestasis must be incorporated into the consideration of the dose of other drugs used in these patients. It also affects the metabolism of other drugs such as anti-cancer drug cyclophosphamide (17), cardiovascular

drug nifedipine (88). The main drugs affected are listed in *Table 1*.

Conclusions

CYP3A4 is an important enzyme in bile acid detoxification, which is responsively up-regulated in cholestasis. Disruption of the CYP adaptive responses in cholestasis leads to more severe hepatic injury, thus retaining of high levels of CYP3A4 protein and its enzymatic activity by CYP3A4 inducers has therapeutic implication. Clinically, it is essential, to reduce the factors which inhibit CYP3A4 and to employ CYP3A4 activators to maximize its activity for detoxification of bile acids in the treatment of cholestasis.

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