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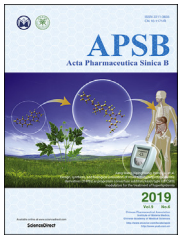

REVIEW

Role of vitamin D receptor in the regulation of *CYP3A* gene expression

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Abstract Vitamin D_3 (VD_3) is a multifunctional nutrient which can be either synthesized or absorbed from the diet. It plays a pivotal role in systemic calcium and phosphate homeostasis, as well as in various physiological and pathological processes. VD_3 is converted to the active form, 1α,25-dihydroxyvitamin D_3 (1,25-D3), by cytochrome P450 2R1 (CYP2R1)/CYP27A1 and CYP27B1 sequentially, and deactivated by multiple enzymes including CYP3A4. On the other hand, 1,25-D3 is capable of activating the transcription of *CYP3A* genes in humans, mice and rats. The vitamin D receptor (VDR)-mediated transactivation of human *CYP3A4* and *CYP3A5* resembles that known for pregnane X receptor (PXR). Activated VDR forms a heterodimer with retinoid X receptor α (RXRα), recruits co-activators, translocates to the cell nucleus, binds to the specific vitamin D responsive elements (VDRE), and activates the gene transcription. In mice, intestinal *Cyp3a11* mRNA levels, but not those of hepatic CYP3As, were induced by *in vivo* administration of VDR and PXR agonists. In rats, intestinal *Cyp3a1* and *Cyp3a2* mRNAs were induced by 1,25-D3 or lithocholic acid (LCA), whereas hepatic *Cyp3a2*, but not *Cyp3a1* and *Cyp3a9*, was modulated to 1,25-D3 treatment. In general, the VDR-mediated regulation of CYP3A presents species and organ specificity.

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1. Introduction

Vitamin D_3 (VD_3), is an important nutrient which can be either synthesized or absorbed from the diet. Traditional roles for VD_3 are the maintenance of calcium and phosphate homeostasis. Mechanisms for regulating intestinal calcium absorption and renal reabsorption are well understood[1]. In positive dietary calcium balance, VD_3 mediates systemic calcium absorption through intestinal epithelial calcium channels expressed on the brush border membrane. Well-known examples include transient potential vanilloid type 6 (TRPV6) and calbindin-D_{9k} channels. Serum calcium is largely responsible for the mineralization of bone matrix. In negative calcium balance, the osteoclast calcium reabsorption is repressed and bone calcium is released into the blood stream to rectify hypocalcemia. The VD_3-facilitated transcellular transport processes of phosphate are similar to those of calcium, and are controlled by sodium-dependent phosphate co-transporters such as sodium-dependent phosphate co-transporter 2b (NPT2b)[2,3]. Global vitamin D receptor (*Vdr*) knockout (KO) mice developed abnormalities including hypocalcemia, secondary hyperparathyroidism and hypophosphatemia after weaning due to the impaired 1α,25-dihydroxybvitamin D_3 (1,25-D3)-dependent intestinal calcium transport. These symptoms were further resolved after the local knock-in of *Vdr* in intestinal epithelial cells[4]. The kidney is another traditional target organ for VD_3. Renal calcium reabsorption in the distal tubules and phosphate reabsorption in the proximal tubules are also regulated by 1,25-D3[1]. Apart from the intestine and kidney, the direct functions of VD_3 on bone are controversial. Although one perspective views of this signaling as "redundant"; another suggests that VD_3 stimulates bone formation and mineralization in human osteoblasts *via* several cellular signaling pathways[4,5].

In recent decades, pleiotropic functions of VD_3 (including its active metabolites, collectively mentioned as VD_3) in physiological and pathological conditions have been gradually revealed. This has occurred in part due to increased understanding of the nuclear receptor VDR. Expression profiling shows that VDR is present not only in classical VD_3-target organs (intestine, kidney, bone, parathyroid glands, etc.) but also in many other cells with diverse derivations[4]. Newly-discovered VD_3 functions indicate its participation in the progression of diverse diseases including metabolic syndromes[6,7], infections[8], cardiovascular diseases[9], cancers[10,11], and even central nervous system disorders[12,13]. VD_3 exhibits diverse effects in animals and humans because it participates in a plethora of biological processes such as proliferation, inflammation, and metabolism[1,14]. Moreover, the fundamental VDR-mediated pathway has become recognized in the regulation network of drug metabolism enzymes.

Xenobiotic biotransformation mechanisms are critical for inactivation and disposal of both externally-ingested drugs as well as endogenous substances. Orally-administered drugs commonly undergo the processes of absorption, distribution, metabolism and excretion *in vivo*[15]. Cytochrome P450 (CYP) enzymes, consisting of several subfamilies and further divided into isoforms, are responsible for the metabolism of most drugs. Human CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 participate in about 90% of known phase I drug metabolic reactions[16]. Among these CYPs, CYP3A4 is the most abundant isoform in human liver and intestine, and plays significant roles in the biotransformation of the greatest number of endobiotics and xenobiotics[17]. CYP3A4 expression and activity are strictly regulated by transcription factors and upstream nuclear receptors, among which the most extensively studies are pregnane X receptor (PXR) and constitutive androstane receptor (CAR). PXR and CAR ligands are largely exogenous compounds, indicating complex interaction between xenobiotics and the body[18]. On the contrary, VDR, which regulated by levels of endogenous ligands including VD_3, can control homeostatic CYP3A4 activity. Because VD_3 can serve as both an endogenous signaling molecule and a nutrient, its bioavailability is subject to complex regulation, with further impaction for the transcriptional activities of *CYP3A* genes[19].

In this review, we first introduce the *in vivo* metabolism profile of VD_3, and the mediation of *Cyp3a* gene transcription by PXR and CAR in humans, mice and rats. We then focus on the species-specific VDR-dependent regulation of human (CYP3A4, CYP3A5 and CYP3A7), mouse (mainly CYP3A11 and CYP3A13), and rat (mainly CYP3A1, CYP3A2 and CYP3A9) CYP3A isoforms (Table 1). In particular, these relationships and mechanisms may help us understand the intra- and inter-individual deviation of human CYP3A4 expression levels, and partly explain the important phenomena of varied oral drug bioavailability.

2. Biotransformation of VD_3

VD_3 can be supplied by either diet or endogenous biosynthesis. 7-Dehydrocholesterol, an intermediate in cholesterol biotransformation, is converted to VD_3 in the skin by exposure to ultraviolet B (UVB) in the sunlight. VD_3 is then converted to 25-hydroxyvitamin D_3 in the liver, by several CYP enzymes including CYP2R1, CYP27A1, CYP2D25, CYP2J2, and CYP3A4. As a major circulating form of VD_3, 25-hydroxyvitamin D_3 is transported to the kidney by vitamin D binding protein. In the proximal renal tubule, 25-hydroxyvitamin D_3 is further catalyzed to 1,25-D3, the most active form of VD_3, by CYP27B1. 1,25-D3 can be deactivated through the metabolism by CYP24A1 to 1,24,25-trihydroxyvitamin D_3 or 1,23,25-trihydroxyvitamin D_3 in the kidney, and is finally oxidized to calcitroic acid by the same enzyme. Furthermore, CYP24A1 is also responsible for the hydroxylation of 25-hydroxyvitamin D_3[1,38]. Recently, the functions of other CYPs (*e.g.*, CYP11A1) in VD_3 biotransformation have been identified, further enhancing our knowledge of VD_3 metabolism[14,39]. Besides phase I metabolism, VD_3 and its metabolites also undergo phase II conjugation. For example, 1,25-D3 is able to be glucuronide-conjugated at the 25-hydroxyl position, mainly by UDP-glucuronyl transferase (UGT) 1A4 and to a less extent by UGT2B4 and UGT2B7. The conjugates are excreted with the bile into intestine, and further re-absorbed into the enterocytes[40].

Human CYP3A4 is highly expressed in the liver and intestine, where VD_3 exercises its main functions by regulating calcium absorption. Although CYP3A4 is not previously identified as the major enzyme responsible for the activation or deactivation of VD_3, its catabolic activity towards VD_3 and its hydroxylated metabolites has been increasingly revealed[41,42]. The CYP3A4 metabolites have been previously designated as "inactive" and CYP3A4-mediated metabolic processes are regarded as "deactivation". However, some physiological effects, especially the anti-tumor activities of CYP3A4 metabolites, support a broader view for the protective and regulatory effects of VD_3 *in vivo*[42]. In humans, CYP3A4 catalyzes the 24- or 25-hydroxylation of 1-hydroxyvitamin D_3, the 23- or 24-hydroxylation of 1,25-D3, and the 4β-hydroxylation of 25-hydroxyvitamin D_3[41,43]. These reactions mainly occur in the liver and/or the intestine, and the concentration of the product, 4β,25-dihydroxyvitamin D_3, is equal to that of 1,25-D3 in plasma[19]. Previous studies reported that in

Table 1 A summary of differentiated responses to VDR ligands by different organs and species.

Species	Organ	CYP3A isoform	1,25-D3 treatment	Ref.	LCA treatment	Ref.
Human	Liver	3A4	↑[a]	20	↑[b]	22,23
			−[b]	21		
		3A5	−	21	＼	
		3A7	−	21	＼	
	Intestine	3A4	↑	24,25	↑	22,26
		3A5	−	20,27	＼	
		3A7	−	20,27	＼	
	Prostate	3A4	↑	28	＼	
		3A5	↑	28	＼	
		3A7	−	28,29	＼	
		3A43	↑	29	＼	
Mouse	Liver	3A11	↑	30	↑	23
	Intestine	3A11	↑	30,31	↑	23,30
Rat	Liver	3A1	−	32,33	−	34
		3A2	↑	32,33	−	34
		3A9	−	34,35	↑	34,35
	Intestine	3A1	↑	33	↑	33
		3A2	↑	33	↑	33
		3A9	−/↑[d]	35−37	↑[c,d]	34

↑ Up-regulated after treatment; − unaffected after treatment; ＼ not studied.
[a] Primary hepatocytes.
[b] Hepatocarcinoma cell lines.
[c] PXR-mediated.
[d] Uncertain.

human hepatic and intestinal microsomes, the 23- or 24-hydroxylation rates of 1,25-D3 were highly correlated with that of midazolam 1′-hydroxylation, and were significantly inhibited by ketoconazole[44]. Therefore, long-term use of some antiepileptic drugs might cause CYP3A4 induction as well as increased turnover of systemic VD_3, and the phenomenon of negative bone mineral balance[44]. Similar effects were also observed after PXR agonist treatment on LS180 cells derived from human colon adenocarcinoma[45]. In contrast, CYP3A4 inhibitors including chemicals and herb monomers inhibited the biotransformation of 1,25-D3 to 1,23*S*,25-trihydroxyvitamin D_3 and 1,24*R*,25-trihydroxyvitamin D_3[43,46].

3. Regulatory roles of PXR and CAR on CYP3A in different species

Human CYP3A4 induction by xenobiotics and hormones has been studied for many years. It is commonly believed that its regulation is involved with PXR (NR1I2), CAR (NR1I3), VDR (NR1I1), glucocorticoid receptor-α (GRα, NR3C1), hepatocyte nuclear factor-4α (HNF4α, NR2A1), HNF3γ, CCAAT/enhancer-binding protein α (C/EBPα) and C/EBPβ in the liver[47,48]. Among these nuclear receptors involved, the interplay between PXR and/or CAR and *CYP3A* genes has been most extensively explored. The complexity of PXR- or CAR-mediated CYP3A induction lies in the broad panel of their ligands, which consist of endogenous steroids and exogenous chemicals. It is noteworthy that some ligands exhibit species selectivity, thus their affinities for the receptors vary across species[18]. For instance, the human PXR (hPXR) agonist, rifampin (RIF), was unable to bind rat or mouse PXR[18,49]. This was verified by the up-regulation of CYP3A11 expression in *PXR/CAR* double humanized mice after RIF treatment, while the hepatic CYP3A11 activity remained unchanged in normal mice[50]. On the contrary, pregnenolone 16α-carbonitrile (PCN) was a selective mouse PXR (mPXR) or rat PXR (rPXR) agonist[51]. Similar phenomena were also observed for CAR ligands. For example, 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl) oxime (CITCO) selectively bound human CAR (hCAR)[52], while 1,4-bis[2-(3,5-dichloropyridyl-oxy-)]benzene (TCPOBOP) only activated mouse CAR (mCAR) but not hCAR[53].

In untreated rodent or human hepatocytes, PXR or CAR is stabilized by cytoplasmic co-chaperone partners like heat shock protein 90 (HSP90, Fig. 1). Upon ligand binding, the nuclear receptors are freed and translocated into the nucleus, identified as a pivotal step in PXR- or CAR-mediated transactivation[15]. The regulation of PXR on CYP3A transcription can thus follow these steps: the binding of ligand to the receptor, the formation of a heterodimer with the retinoid X receptor α (RXRα), the recruitment of co-activators (for example, the steroid receptor coactivators 1, SRC-1), the binding to PXR-response elements (PXREs), and the transcriptional regulation of target genes. The DNA response elements include direct repeats (DR)-3, DR-4, DR-5 and everted repeats (ER)-6 and ER-8. PXR agonists robustly activated the transcription of *Cyp3a* genes in different species, while compounds might suppress the constitutive and inductive CYP3A4 expression by interfering the processes in PXR-mediated transcription[54,55]. The transactivation process by CAR is similar, except for the binding of CAR-RXRα complex to the phenobarbital responsive enhancer modules (PBREM) of the target genes[18,49]. The binding to PBREM was specially discovered in the regulation of human *CYP2B6* or mouse *Cyp2b10* genes, but it was reported that human CYP2B6 and CY3A4 shared crossed substrate specificity and regulation networks[56]. CAR was also found to transactivate the steroid/RIF-responsive ER6 motif of the human *CYP3A4* gene[57]. These facts indicate the versatility of PXR and CAR in the regulation of target genes to some extent.

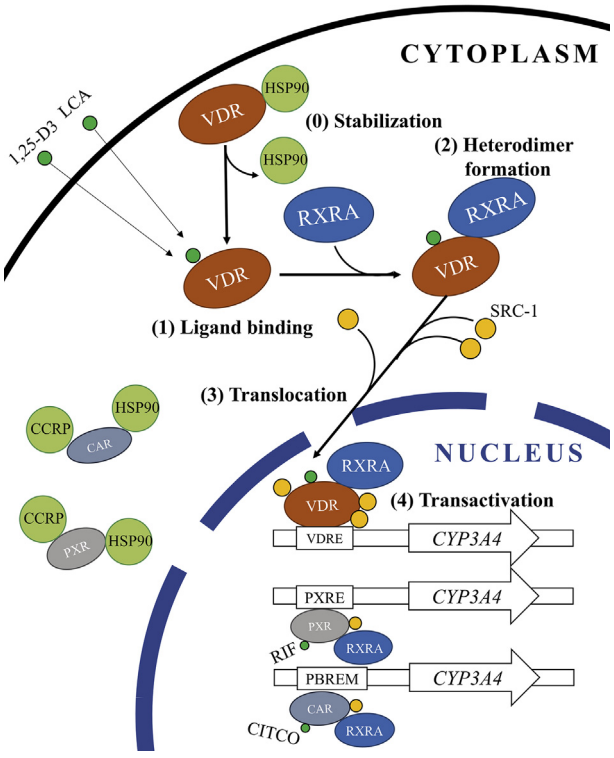

Figure 1 Vitamin D receptor (VDR)-mediated transactivation of *CYP3A4* gene. When it is not bound with ligands, VDR is retained with chaperone proteins (for instance, the heat shock protein 90, HSP90) in the cytoplasm. Activated by ligands like 1,25-dihydroxyvitamin D_3 (1,25-D3) or lithocholic acid (LCA), VDR forms a heterodimer with retinoid X receptor α (RXRα), recruits co-activators (including, but not restricted to the steroid receptor coactivators 1, SRC-1), translocates into the nucleus, binds to the vitamin D response element (VDRE) of *CYP3A4* promoter, and up-regulates *CYP3A4* transcription. Human pregnane X receptor (PXR) and constitutive androstane receptor (CAR) transactivates *CYP3A4* in a similar manner, except for different ligands (rifampin, RIF, for PXR and 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime, CITCO, for CAR) and response elements in the gene promoter (PXR-response element, PXRE, for PXR and phenobarbital responsive enhancer modules, PBREM for CAR).

The inductive effects of PXR or CAR ligands depend on the organ-specific expression of the nuclear receptors. PXR is primarily expressed in rodent and human livers, as well as human testis and mouse intestine. CAR is abundant in rodent and human liver and kidney, and is also detected in human brain or mouse intestine[18]. The regulatory effects of PXR and CAR agonists on human *CYP3A4* are overlapping but biased. Although both RIF and CITCO are able to significantly activate the transcription of *CYP3A4* and *CYP2B6* in human primary hepatocytes, comparatively, RIF is more effective in *CYP3A4* induction, and CITCO is a more potent inducer of the latter gene[52]. Treatment with RIF significantly increased *CYP3A4* mRNA levels and CYP3A4 catalytic activities in Caco-2 and LS-180 cells, which lead to the elevated catabolism of many active endogenous substances including 1,25-D3[45].

For mouse, PCN was capable of inducing *Cyp3a11* transcription in both liver and intestine, while TCPOBOP only induced hepatic *Cyp3a11* mRNA levels. These effects were not observed in *Pxr*- or *Car*-null mice[58]. In rat hepatic slices, *Cyp3a1* was induced by the PXR ligand, PCN, and the PXR/GR ligand, dexamethasone; *Cyp3a2* transcription was suppressed by the GR ligand, budesonide; *Cyp3a9* was induced by all of the three ligands[34]. On the contrary, the involvement of CAR in the induction of rat CYPs has rarely been studied. Besides the phase I metabolic enzymes, PXR and/or CAR also participates in the regulation of mouse hepatic phase II metabolic enzymes (UDP-glucuronosyltransferases, *Ugt1a1*; sulfotransferases, *Sultn*; glutathione *S*-transferases, *Gsta1*, *Gstm1*, *Gstm2* and *Gstt1*) and transporters (multiple drug resistance gene, *Mdr1a*, *Mdr1b*; multidrug resistance-associated protein, *Mrp2* and *Mrp3*) in distinct but overlapping manners[58].

4. Regulatory roles of VDR on CYP3A in different species

4.1. Human

4.1.1. Mechanism of VDR-mediated human CYP3A transactivation

The regulatory effects of VDR on human CYP3As are believed to share common mechanisms with those of PXR (Fig. 1). The high similarity in the DNA-binding domains (64%) between PXR and VDR indicates overlapping transactivation motifs. On the other hand, the relative low similarity of their ligand-binding domains (37%) accounts for the discrepancies in their activating modes[59]. It was assumed that the extremely large binding pockets of PXR and VDR observed in X-ray crystallographic assays enabled them accessible to ligands of diverse structures[26]. Along with the flexibility in binding diverse DNA motifs, the nuclear receptors might regulate the transcription of an enormous panel of genes[60]. VDR is located in the cytosol of VD_3-sensitive cells. When VDR is bound by 1,25-D3, the complex forms a heterodimer with RXR, translocates to the cell nucleus, binds to the specific DNA-sequences referred to as vitamin D responsive elements (VDRE), and activates or represses the transcription of related genes[1,38]. VDR and PXR also share similar responsive elements in the *Cyp3a4* gene promoter such as ER6, DR3 (dNR1), DR4 (eNR3A4)[28,61]. From the experiment results of human intestine- or colon-derived cell lines (for instance, Caco-2 and LS180), two transactivation motifs were identified: the distal DR3 motif and the proximal DR6 motif, which were essential for the induction of this gene by 1,25-D3[62]. The two relevant VDREs consist of distinctly diverse DNA motifs and are far separated in the promoting sequence of the gene. The distal DR3 motif is similar to the classic elements in those 1,25-D3 targeting genes, and is localized almost 8000 bp upstream of the starting site of *CYP3A4* transcription[26].

The sequence conservation of both the distal DR3 motifs and the proximal DR6 motifs in the *CYP3A4* promoter is pivotal to the transactivation functions of VDR-RXR on CYP3As[62]. *CYP3A4* and *CYP3A5* are highly related in genes sequences (89%). *CYP3A5*, however, lacks a distal xenobiotic-responsive enhancer module (XREM) region (−7836 to −7208 bp) containing ER6 and DR3 motifs. It was able to bind HNF4α compared with *CYP3A4* and *CYP3A7*[63,64]. This dissimilarity may be the reason for the differential regulatory mechanisms between *CYP3A4* and *CYP3A5*. For the induction of *CYP3A5*, some studies mentioned the involvement of GR, PXR and CAR in the liver and intestine[65]. Others pointed out that neither its basal expression in extrahepatic

tissues was dependent on PXR, nor its transcription was responsive to PXR ligands[66]. Neither the hepatic nor intestinal *CYP3A5* genes were sensitive to VDR ligands (discussed in next sections). Levels of CYP3A7, a human fetal form of hepatic CYP3A that decline along with the increased expression of CYP3A4[65], were not induced by 1,25-D3. Comparisons of the proximal ER6 motif of *CYP3A7* with that of *CYP3A4*, found two nucleotide mutations (−169G→−168T, −161A→−160T) and a 10-nucleotide deletion corresponding to the region between −295 and −286 bp of *CYP3A4* proximal promoter[62]. These two mutations, but not the deletion, may account for the fact that PXR-RXRα and VDR-RXRα are no longer able to recognize and bind the promoter sequence of *CYP3A7* and activate the transcription of this isoform[21,62]. In fact, a *CYP3A7* gene polymorphism (*CYP3A7*1C*) has been identified with high CYP3A7 expression in adult liver and intestine. *CYP3A7* and *CYP3A7*1C* allele carriers had differential sequences in the ER6 motif of the gene promoters[67]. The nucleotide sequence of the latter (identical to that of *CYP3A4*) made it sensitive to PXR-mediated promoter activation, and probably also susceptible to VDR-mediated transactivation[62]. *CYP3A43* was the fourth member of human *CYP3A* genes, with 75.8% amino acid sequence identity to *CYP3A4*. Highest expression of *CYP3A43* mRNA is in the prostate, with detectable levels in the brain, placenta, liver, and testis[68,69]. Moreover, *CYP3A43* was detected in all Caucasian liver samples, but its levels varied up to 1000-fold. In human primary hepatocytes, *CYP3A43* was also susceptible to RIF induction[68,70]. However, the responses of *CYP3A43* to some other known *CYP3A4* inducers were differentiated[71]. Little is known yet about the metabolic capacity of *CYP3A43* on VD_3, but the substrate selectivity between CYP3A4 and CYP3A43 is substantial.

The relationship between PXR and VDR on CYP3A4 induction is controversial. For example, one study showed that VDR and PXR synergistically cooperate in the transactivation of CYP3A4 reporter in an intestinal cell line, LS174T[61], whereas another study contrastingly reported competition between VDR and PXR on the regulation of CYP3A4 in HepG2 cells. The effects of PXR on *CYP3A4* seemed to predominate over those of VDR when the two plasmids were co-transfected and the two ligands (RIF and 1,25-D3) were simultaneously given[72]. Considering the different resources of the cell lines used in the two studies, the controversy might be explained. As it will be discussed in the next section, PXR was predominantly expressed in the liver, while VDR levels were higher in the intestine. However, high basal expression level of VDR was detected in LS174T[61], while the authors suggested low endogenous level of PXR and VDR in HepG2 cells[72]. Therefore, the interaction between the two nuclear receptors on the regulation of CYP3As could be tissue-specific and ligand-dependent, but also be associated with the basal expression profiles of the nuclear receptors. The relationship between PXR and VDR, although poorly understood, may contribute to the complexity of the regulatory network of xenobiotic metabolic enzymes. There is notable overlap in the regulatory functions of PXR and VDR. By sharing the transactivating motifs with PXR, the VDR-RXRα heterodimer was also able to activate the expression of human CYP2B6 and CYP2C9, although the effect on the latter enzyme was more modest[47,72]. For comparison, protein levels of CYP1A1 and CYP2D6 were not influenced by 1,25-D3 treatment[27]. Interestingly VDR also participates in the regulation of phase II metabolic enzymes, such as *UGT2B15/2B17*[29,73] and *SULT2A1*[74]. Furthermore, VD_3 has been shown to up-regulate efflux transporters including *MDR1*, *MRP2*, *MRP3*, as well as *MRP4*[24,74,75], and uptake transporters such as organic anion transporting polypeptide 1A2 (*OATP1A2*)[76], which share same VDREs with *CYP3A4*.

Some studies also disclosed that many protein kinases are involved in the interaction between 1,25-D3 and *CYP3A4*. Thus, 1,25-D3 induced the expression of CYP3A4 by both ligand-dependent and ligand-independent mechanisms. Using specific protein kinase inhibitors, the participation of protein kinase C (PKC), tyrosine kinase, mitogen-activated protein kinases (MAPKs) and c-Jun N-terminal kinase (JNK) was identified, whereas protein kinase A (PKA), extracellular signal-regulated kinase (ERK) and p38 were not relevant[77,78]. The authors suggested that 1,25-D3 activated PKC and JNK through a non-genomic signal pathway, and the activated protein kinases recruited transcriptional factors like AP-1 and Sp-1, assisting the transactivation effects of VDR on *CYP3A4*[78].

4.1.2. *Results from cell lines*

Most studies focus on the effects of VDR on the major human CYP3A isoform, CYP3A4, and are most commonly conducted with intestine and/or liver tissue, in which the expression of CYP3A4 is abundant. In contrast to PXR, VDR recognizes a narrow ligand spectrum, including bile acids and VD_3 (including its metabolites and derivatives)[21]. The affinity of the ligands for VDR follows the rank order: 1,25-D3>25-hydroxyvitamin D_3>VD_3, and higher activating capacities of VDR result in stronger CYP3A4 induction[47]. That is, 25-hydroxyvitamin D_3 was able to increase CYP3A4 catalytic activity, although to a less extent compared with 1,25-D3, and the unhydroxylated VD_3 had no effect of inducing CYP3A4 protein expression or activity[27].

Although liver and intestine are both significant in the first-pass metabolism of orally-administered substances, the expression and regulation of nuclear receptors and catabolic enzymes are distinct between these tissues. PXR is abundant in hepatocytes, but is not predominant in the regulation of intestinal CYP3As[18]. On the contrary, VDR is highly expressed in intestine- or colon-derived cell lines, but not in liver-derived cell lines or parenchymal cells[19]. The expression levels of VDR correlates with those of intestinal CYP3A4 which gradually decreases from the proximal small intestine to the colon. The effects of 1,25-D3 on intestinal CYP3A4 were uncontroversial, fast and dose-dependent according to *in vitro* studies. The transcription of *CYP3A4* in intestinal cell lines (LS180 and Caco-2) was induced by 1,25-D3 in nanomolar levels, which necessitated the expression of VDR, but not PXR[24]. *CYP3A4* mRNA amplification occurred only 6 h after 1,25-D3 treatment, and prolonged treatment duration leaded to further increases[25]. CYP3A4 protein levels and catalytic activity were also quickly up-regulated after 1,25-D3, 25-hydroxyvitamin D_3 or 1α-hydroxyvitamin D_3 treatment in Caco-2 cells, but CYP3A5 or CYP3A7 protein levels were not induced by 1,25-D3 in either Caco-2 nor HPAC (pancreatic adenocarcinoma) cell lines[20,27].

In contrast, conclusions on whether CYP3A4 could be induced by 1,25-D3 in hepatic cell lines were inconsistent. For instance, one study reported both basal and calcitriol-induced CYP3A4 expression in the hepatocarcinoma-derived HepG2 cells[79]. On the contrary, another study concluded that HepG2 cells were not a good tool to study CYP3A4 induction, because in this cell line CYP3A7 was expressed instead of CYP3A4, and CYP3A4 was not induced by RIF despite of the presence of PXR. Moreover, *CYP3A4* mRNA levels were also unaffected by 1,25-D3 treatment[21]. The controversial results from these two articles may be

due to the different 1,25-D3 concentrations (100 and 250 nmol/L *vs.* 1 and 100 nmol/L) and/or treatment durations (up to 144 h *vs.* 48 h). Interestingly, different from the nonresponsiveness in HepG2, *CYP3A4* transcription in primary human hepatocytes was susceptible to 1,25-D3 treatment[20]. Khan et al.[34] suggested that VDR might be expressed more abundantly in human hepatocytes compared with rat hepatocytes or human hepatocarcinoma cell lines. Therefore, the differentiated results by known PXR or VDR ligands from HepG2 and human primary hepatocytes may be attributed to the loss of nuclear receptors and the changes of enzyme profiles in the carcinoma-derived cell line.

Although CYP3A4 is mainly expressed in the intestine and liver, its existence and functions in other organs and tissues are gradually being revealed. Extrahepatic CYP3A4 is usually responsible for the *in situ* metabolism of hormones and signal molecules. For instance, CYP3A4 participates in the irreversible oxidation of testosterone and terminates its androgenic effects in prostate[73]. Androgens including androstenedione, dehydroepiandrosterone, testosterone and dihydrotestosterone serve as substrates of CYP3A4. 1,25-D3 treatment significantly increased the gene transcription of *CYP3A4*, *CYP3A5*, *CYP24A1* and *VDR* but not *AR* (androgen receptor) in LNCap or LAPC-4 cells. *CYP3A5* was more responsive to 1,25-D3 than *CYP3A4* in LAPC-4 cells. 1,25-D3 also increased the enzymatic level of CYP3A4 and its catalytic activity (measured by the turnover velocities of testosterone and dehydroepiandrosterone). Enhanced CYP3A4/3A5 activities accelerated the catabolism of testosterone and inhibited cell growth, while in the presence of the CYP3A4 inhibitor, ritonavir, the anti-proliferative effects of 1,25-D3 were partly impaired[28]. In contrast, the expression of CYP3A7 was independent of VDR regulation[28,29]. The synthetic VDR agonist, EB1089, had similar effects on *CYP3A4*, *CYP3A5* and *CYP3A7* genes as 1,25-D3. Surprisingly, *CYP3A43* mRNA was significantly induced by EB1089. The functions of VDR on CYP3A4 in LNCap and LAPC-4 cells seemed exclusive, as the expression levels of PXR and FXR were negligible in these two prostatic cancer cell lines[29]. Therefore, VDR may partly exert its anti-proliferative effects by inducing *in situ* CYP3A4 level and modulating androgen metabolism.

4.1.3. Results from organ slices

CYP3A4, CYP3A5 and CYP3A7 belong to three categories of metabolic enzymes considering their differential expression profiles in fetuses and infants. CYP3A7 is highly expressed in the first trimester in gestation and gradually decreases afterwards. CYP3A5 is steadily expressed until 1−2 years after birth. CYP3A4 protein level is extremely low in the first trimester, but increases quickly in the second and third trimester of pregnancy[80]. Regarding basal CYP3A expression, significant correlations between *VDR* and *CYP3A4*/*3A7* mRNA levels in fetal liver tissue, and between *VDR* and *CYP3A4* mRNA levels in fetal intestine tissue, were established[81]. Another study indicated that 1,25-D3 was as an essential auto/paracrine hormonal factor for the complex regulation of several VD_3-responsive genes and fetal gut development. In both proximal and distal intestine and colon in specimens from fetuses 15−20 weeks after gestation, *VDR* and *CYP3A4* mRNA were detected. *CYP3A4* was greatly induced by 1,25-D3, while the effects also showed significant inter-individual variability. That is, in one of the four specimens, *CYP3A4* mRNA level was not influenced by 1,25-D3 treatment. In addition, there were positive relative relationships between the induction profiles of CYP3A4 and the VD_3 24-hydroxylase CYP24A1[82].

In adults, the enterohepatic circulation of VD_3 and its metabolites may be a factor for the preferential expression of VD_3-target genes in the proximal intestine compared with the distal intestine. Radiolabeled 1,25-D3 and 25-hydroxyvitamin D_3 were discovered in the bile and afterwards re-absorbed into the intestinal epithelial cells. High local concentrations of active VD metabolites efficiently motivated the expression of VD_3-responsive genes such as *CYP3A4* and *TRVP6*[19]. There was no correlation between the basal mRNA levels of *VDR* and *CYP3A4*/*3A7* in adult liver tissues[81], but *CYP3A4* was strongly induced in human ileum slices *ex vivo* by VDR, PXR and GR ligands, which was in accordance with the results observed in human intestinal cell monolayers. Similar results were also obtained in human liver slices, but were restricted to the samples in which VDR was expressed[35].

4.1.4. Results from patients

No apparent correlations have been reported between the intestinal and hepatic CYP3A4 expressions or catalytic activities in individuals. Much higher CYP3A4 levels were found in enterocytes *vs*. hepatocytes, which play a significant role in the gut biotransformation of many substrates[83]. Due to the differential localization of VDR in liver and intestine, the regulation of CYP3A4 expression by VD_3 and VDR may be one of the organ-specific factors. Some studies pointed out that seasonal variation in sunlight exposure and plasma VD_3 levels could theoretically account for fluctuating CYP3A4 levels over the year, and would hence influence drug turnover[38]. For instance, chronically administered CYP3A4 substrates such as the immunosuppressants sirolimus and tacrolimus had significantly lower concentration/dose ratios (−17% and −5%, respectively) during the summer months, compared to those in the winter in Sweden. Comparatively, the CYP3A5-mediated metabolism of these drugs was less influenced by the seasonal variation of UVB exposure and intrinsic VD_3 levels[84]. Abundant evidence verified that pathological conditions were capable of altering metabolic enzyme expression and activity, and hence the *in vivo* profiles of drugs and endogenous substances. In patients with end-stage renal disease, the decrease of 1,25-D3 and the accumulation of uremic toxins contributed to the decreased hepatic clearance of CYP3A4 substrates[85].

In a clinical trial of 87 patients with abnormal glucose homeostasis or diabetes, no significant changes were seen in hepatic CYP3A4 activities (measured as delta serum 4β-hydroxycholesterol/cholesterol ratio), although patients receiving 30,000 IU of oral VD_3 once a week for 8 weeks had increased mean serum concentrations of 25-hydroxyvitamin D_3. Nonetheless, the authors thought it could not exclude the possible alterations in the intestinal metabolism of oral drugs after high doses of VD_3 administration[86]. Atorvastatin, a β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase inhibitor for the treatment of hyperlipidemia, is metabolized to two active metabolites by CYP3A4 *in vivo*, which are then further deactivated by CYP3A4. In a clinical trial of 16 patients, the group taking atorvastatin along with VD_3 supplements showed lower bioavailabilities of atorvastatin and its metabolites. The lower total area under the curve (AUC) exhibited a trend (not significant) in correlation with higher active VD_3 plasma concentrations[87].

In conclusion, despite of insufficient clinical data, further studies are needed to establish relationships between *in vivo* CYP3A4 activity and substrate bioavailability, as well as between *in vivo* CYP3A4 activity and the elimination rates and systemic VD_3 levels influenced by seasonal, pathological or pharmacological factors.

4.1.5. Other VDR ligands

Apart from VD_3 and its mono-, di- and tri-hydroxylated metabolites and analogs, a carcinogenic secondary bile acid, lithocholic acid (LCA), also serves as an endogenous VDR ligand. Mutagenesis assays in the ligand binding domain of VDR indicated that S237 and S225/S278 were critical for 1,25-D3 and LCA action, respectively[22]. LCA is transformed by intestinal bacteria from the primary bile acid, chenodeoxycholic acid (CDCA)[88]. Interestingly, although primary bile acids only showed affinity for farnesoid X receptor (FXR) but not VDR, LCA (as well as its major metabolites) could activate both VDR and FXR[89]. The LCA-VDR complex was also able to combine and activate the ER6 and DR3 motifs and induce the expression of CYP3A4 in HT-29 (a colon cancer cell line), although it was not as effective as 1,25-D3 at the same concentration. The amplification effects correlated with the expression levels of VDR[22,26]. Similar effects of LCA and 1,25-D3 in activating VDR were also observed in HepG2, LST174, kidney (monkey COS-7 cell line and human HEK-293 cell line) and rat osteoblast-like osteosarcoma cell lines[22,23]. The inductive effects of LCA were observed at lower concentrations and higher extents in LST174 cells compared with HepG2 cells. These effects were believed to work through the VDR-mediated pathway rather than the PXR-mediated pathway as they could be attenuated by the introduction of small interference RNA (siRNA) of *VDR*[23]. Another reason of focusing on VDR rather than PXR was that the EC_{50} values of either LCA or 3-keto-LCA (the metabolite of LCA) on PXR were 10 times higher than those on VDR. Therefore, the concentration of LCA used in the aforementioned experiments was insufficient to activate PXR[30]. Transfection of exogenous *CYP3A4* VDRE, RXR or SRC-1 into HT-29 cells seemed to enhance the effects of LCR−VDR complex more effectively compared with 1,25-D3-VDR complex[22].

In human ileum slices, LCA (10 μmol/L) significantly increased *CYP3A4* expression in a time-dependent manner (accompanied with changes of *MRP2* and *MRP3* levels), although the effects were not consistent across all of the human liver slices[34]. Conclusively, as a cytotoxic metabolite of CDCA produced by gut bacteria, LCA up-regulated the expression of CYP3A4 in both small and large intestines, promoting the detoxification of itself and reducing the risks of colon cancer[22]. The inductive effects of LCA on *CYP3A4* gene was called a "feed-forward mechanism" as CYP3A4 efficiently 1β-hydroxylated LCA and prevented its accumulation. CYP3A4 is also capable of transforming LCA to 3-keto-LCA, a more potent ligand for PXR and VDR and regulator on CYP3A4 expression[59,90]. Thus the constructive expression of CYP3A4 in enterocytes may be maintained by natural 1,25-D3 that efficiently binds to VDR, and *CYP3A4* transcription may be additionally elevated by high local concentrations of LCA to benefit the detoxification of this carcinogen[26].

There is a sustained medicinal chemistry effort to synthesize VDR ligands based on the crystal structure of this nuclear receptor, and to create analogs with a broad spectrum of physiological impact[91]. At the same time, agonist effects of some natural compounds on VDR are discovered, though these compounds are not similar to VD_3 in their chemical structures. Flavonoids are abundant in fruits, vegetables and beverages. Several studies suggest that some flavonoids also showed affinity towards VDR at relatively high concentrations. For instance, curcumin was identified as a low-affinity ligand for VDR *via* several assays. In particular, 10 μmol/L of curcumin resulted in equal effects on the transcription of the VDRE-reporter plasmid compared with 10 nmol/L of 1,25-D3. Curcumin was also capable of competing with 1,25-D3, binding to VDR directly and recruiting co-modulators like RXRα and SRC-1, and thus transactivating VDR-target genes, including *CYP3A4*[92]. Another study found that 3-day treatment of 50 μmol/L quercetin on Caco-2 cells slightly but significantly up-regulated *VDR* and VDR-target genes, including *CYP24A1*, *CYP3A4*, *MDR1* and *TRPV6*, and the correlated higher expression of CYP3A4 and P-gp proteins were observed. Introduction of *VDR* siRNA suggested that the effects of quercetin on CYP3A4 required the normal functions of VDR in Caco-2 cells. Other quercetin-like flavonoids (kaempferol and berberine) of equal concentration and treatment duration resulted in similar effects on these genes[93]. The inductive effects of complementary and alternative medicines (CAM) on CYP3A4 through PXR- or CAR-mediated pathway has been elucidated[94]. It hence seems that VDR also plays an essential role in the interplay between flavonoids and CYP3A4. Although these compounds present relative weaker VDR affinity compared with 1,25-D3, dietary intake can easily form a local environment with high flavonoid concentrations (micromolar levels) in the intestine. This raises concerns on diet-drug interactions, and whether long-term flavonoids intake will enhance VD_3 catabolism from induced CYP3A4 expression.

4.1.6. Gene polymorphism

The phenomenon of *VDR* gene polymorphism is frequently observed in clinic[14]. One set of *VDR* single nucleotide polymorphism (SNP) (Fok1 and EcoRV) is localized near its 5′-end, another (*Taq*I, *Bsm*I and *poly*A) is near the 3′ untranslated region. The Fok1 polymorphism, for example, results in a shorter VDR protein sequence[95]. The inductive effects of 25-hydroxyvitamin D_3 on CYP3A4 may be largely confined to rs4516035 (GATA-1012A > G) AG/AA carriers, but the basal CYP3A4 activity in rs4516035 GG carriers is high in spite of low serum 25-hydroxyvitamin D_3 levels, and does not increase further with elevated 25-hydroxyvitamin D_3 concentrations[96]. Thirumaran et al.[97] reported no association between *VDR Bsm*I polymorphism (rs1544410, intron 8 *Bsm*I-G>A) and hepatic CYP3A4 protein expression or intravenous midazolam clearance as VDR was not sufficiently expressed in human hepatocytes. However, *VDR Bsm*I-G genotype was associated with higher intestinal CYP3A4 expression/activity quantified by oral midazolam clearance in Caucasian populations. The authors supposed that this *VDR* allele possessed a GATA binding site in *VDR* promoter that transactivated *VDR*, and thus induced the expression of CYP3A4 in intestine[97].

There is bidirectional regulation between VDR and CYP3A4. The major CYP3A4 allele rs2242480 (in intron 10, near the exon/intron boundary) is associated with higher serum 25-hydroxyvitamin D3 concentrations among middle-aged and elderly Chinese in Singapore. Although the functional relevance of this SNP is unclear, a recent pharmacokinetic study suggests that individuals with the homozygous variant rs2242480 TT genotype have significantly lower CYP3A4 activity[98]. This correlation might indicate the significance of CYP3A4 function in maintaining integrates 25-hydroxyvitamin D_3 levels, but the relationship and physiological outcomes needed more detailed research.

In short, we can partly conclude that factors like individual dietary habits and/or duration/intensity of sun exposure, might contribute to the intra- and inter-individual diversity of CYP3A4 expression. More importantly, when systemic or local VD_3 levels

fluctuate, the disposition of the drugs that serve as CYP3A4 substrates may be changed[72]. VDR-mediated *CYP3A4* induction could bring complex physiological and pharmacological consequences in at least two aspects. If drugs subject to CYP3A4 metabolism are taken along with VDR modulators or under pathological conditions (for instance, during cholestasis, LCA level was significantly increased), their bioavailability and disposition could be changed, and the potential for drug−drug interactions (DDI) should be carefully considered[19,34]. On the other hand, as 1,25-D3 is not only an inducer but also a substrate of CYP3A4, enhanced 1,25-D3 catabolism in CYP3A4-abundant tissues (for example, the intestine) would influence the downstream regulation of apical membrane calcium transport protein expression, like TRPV6, and possibly impair the intestinal calcium absorption[45].

4.2. Mouse

The expression of five CYP3A proteins (CYP3A11, CYP3A13, CYP3A16, CYP3A25 and CYP3A44) was studied in mouse intestinal epithelial cells. Measured by mRNA levels, *Cyp3a13* was extensively transcribed in the intestine, while *Cyp3a41* was most abundant in the liver[99]. In mouse liver, CYP3A13 was constitutively expressed, but was not an inducible isoform. In contrast, CYP3A11 was barely detected in liver homogenate until induced by, for instance, phenytoin. Similarly, in mouse brain, the expression of CYP3A13 was beyond the detection limit, while CYP3A11 was significantly induced by phenytoin treatment[100,101]. Although VDR protein levels were much higher in mouse hepatocytes as compared with human or rat hepatocytes, VDR expression was still lower than in mouse duodenocytes[102]. Another study reported detection of mouse PXR in both mice liver and intestine, yet VDR was mainly present in the latter organ[23]. VDR was also abundantly expressed in mouse kidney[103].

Mouse intestinal *Cyp3a11* mRNA level was responsive to the *in vivo* administration of VDR (1α-hydroxyvitamin D_3 and LCA) and PXR ligands. The induction of *Cyp3a11* mRNA by LCA was independent of PXR validated by *Pxr* knockout mice[30]. In *Pxr*/*Car* double null mouse hepatocytes, PXR (PCN) or CAR (TCPOBOP) agonists no longer triggered the transcription of *Cyp3a11*, but 1,25-D3 still induced *Cyp3a11* mRNA level[31].

Increased *Cyp3a* mRNA levels were detected in the intestine but not in the liver of mice orally receiving 100 mg/kg/day LCA for 3 days. However, when mouse livers were transfected with human VDR adenovirus, the hepatic CYP3A expression was also induced by LCA. This result indicates that the inductive effect of LCA in mice is dependent upon VDR[23].

The bidirectional modulation between LCA and human intestinal CYP3A4 is well established. As known in the case of humans, LCA induces mouse *Cyp3a11*, and yet mouse intestinal CYP3As also play pivotal roles in detoxifying LCA. Cheng et al.[104] used a panel of mouse models to study LCA-caused hepatotoxicity. Toxicity was measured as increased alanine aminotransferase (ALT) and alkaline phosphatase (ALP) values in wildtype (WT) mice, mice with intestine-specific disruption of VDR mice ($Vdr^{\Delta IEpC}$), mice with transgenic-CYP3A4 (Tg-3A4), and mice with both intestine-specific disruption of VDR and transgenic-CYP3A4 ($Vdr^{\Delta IEpC}$/3A4). The severity of injury followed the order: $Vdr^{\Delta IEpC}$>WT, $Vdr^{\Delta IEpC}$/3A4>Tg-3A4. This order suggests that LCA hepatotoxicity can be aggravated in the absence of intestinal VDR, and may be alleviated by the genetic insertion of human *CYP3A4*. The authors also suggested that, in $Vdr^{\Delta IEpC}$ mice, the knockout of intestinal *Vdr* might result in the damage of intestine permeability, with an ensuing increase in the re-uptake of bile acids into the hepatocytes. Cholesterol and bile acids were thus more synthesized and accumulated in hepatocytes, which lead to more severe LCA toxicity. The excreted intestinal bile acids were metabolized by intestinal-specific expression of CYP3A4 in $Vdr^{\Delta IEpC}$/3A4 mice, and rescued the hepatocytes from excessive bile acid exposure[104].

4.3. Rat

Among the several rat CYP3A isoforms, CYP3A9, CYP3A18 and CYP3A62 have been detected in the intestine, whereas CYP3A1 and CYP3A2 are the main hepatic isoforms[99]. Although the constitutive expression of CYP3A1/23 (mentioned as CYP3A1 in following discussion) (https://www.ncbi.nlm.nih.gov/gene/25642) was beyond detection limit in the intestine, it served as a main inducible isoform of CYP3A in rat intestine and liver[105]. VDR is highly expressed in rat intestinal epithelial cells, while in liver, mRNA or protein of VDR is mainly detected in nonparenchymal cells (sinusoidal endothelial, Kupffer, stellate, and biliary epithelial cells) rather than hepatocytes[33,102]. Except for the similarity in the preferential expression of VDR in intestinal mucosal epithelial cells *vs*. hepatocytes, rat *Cyp3a1* and human *CYP3A4* share common VD_3-responsive transcriptional enhancer elements[105]. CYP3A1 is equally effectively induced by LCA and 1,25-D3, with the participation of *Cyp3a1* DR3 as a VDRE[22]. Compared with human *CYP3A4* DR3, the rat *Cyp3a1* PDR3 is localized much further upstream toward the gene[26]. However, the mouse or rat *Cyp3a* genes lack a proximal DR6 element similar to that of human *CYP3A4*[26,30].

4.3.1. CYP3A1 and CYP3A2

The inductive effects of 1,25-D3 on rat CYP3As are dose-dependent, isoform-dependent and region-dependent. In the proximal rat intestine, *Cyp3a1* instead of *Cyp3a2* mRNA has been abundantly detected, but the induction of *Cyp3a2* was still observed after VDR ligand treatment. Incubation of 1,25-D3 with the precise slices of rat intestine significantly induced *Cyp3a1* mRNA levels, while the inducing effects differentiated among colon, ileum and jejunum. *Cyp3a2* mRNA was highly induced after incubation with 1,25-D3 in ileum slices, but not in jejunum and colon slices[32]. Intraperitoneal treatment of 1,25-D3 (100 ng) significantly and selectively elevated CYP3A1 mRNA and protein levels in male rat intestine. Apart from 1,25-D3, 8 h incubation with LCA (10 μmol/L) or CDCA (50 μmol/L) also induced *Cyp3a1* mRNA in rat ileum slices, yet *Cyp3a2* mRNA was only induced in 1,25-D3 or LCA groups. The inductive effects of 1,25-D3 and LCA on *Cyp3a1* and *Cyp3a2* were attenuated by CDCA in a dose-dependent manner (1-50 μmol/L)[33].

In rat liver, VDR was only detected in cholangiocytes, where CYP3A2, but not CYP3A1 and CYP3A9, co-exist. Therefore, although hepatic *Vdr* mRNA level was significantly increased after 1,25-D3 treatment, only the mRNA level of *Cyp3a2* (in cholangiocytes), but not *Cyp3a1* (in hepatocytes), was simultaneously up-regulated[32,33]. Hepatic *Cyp3a1* and *Cyp3a2* transcription were not affected by LCA incubation[34]. Taking P-gp expression into consideration, experiments utilizing a dual substrate of P-gp and CYP3A showed the failure of VDR-mediated regulation on rat hepatic CYP3As. 1,25-D3 changed the *in vivo* distribution of quinidine by up-regulating the cerebral P-gp expression, but failed to alter its systemic clearance or turnover

rate in liver microsomal incubation[106]. Hepatic CYP3A protein levels were not changed after 1,25-D3 treatment[106].

4.3.2. *CYP3A9*

For CYP3A9, the effects of 1,25-D3 treatment were not consistent. The transcription of *Cyp3a9* in rat colon, ileum or jejunum slices was strongly up-regulated by PCN, budesonide (a GR ligand) and dexamethasone (a PXR/GR ligand)[35], but less sensitive to PXR-mediated induction compared with *Cyp3a1*[105]. On the contrary, some studies concluded that *Cyp3a9* was not induced by 1,25-D3, in either *ex vivo*[35] or *in vivo*[32,105] experiments. The same laboratory, however, reported an approximate 2-fold increase of *Cyp3a9* mRNA levels in rat ileum, along with highly variable but significant increases in this mRNA from rat liver and kidney following repeated intraperitoneal dose of 2.56 nmol/kg or 6.4 nmol/kg 1,25-D3; *Cyp3a1* mRNA levels remained unchanged[36,37]. The authors did not explain the discrepancy in results concerning *Cyp3a9*, but this might result from the different doses of 1,25-D3 used in the two experiments. In precise-cut slices from male rat jejunum, ileum and colon, 10 μmol/L of LCA modestly induced *Cyp3a9* mRNA levels. However, the inductive trends of LCA from jejunum to colon did not resemble the relative abundance of VDR in the different parts. The authors presumptively attributed the induction of *Cyp3a9* to the interaction between 3-keto-LCA and PXR, instead of LCA or VDR, as 1,25-D3 failed to induce *Cyp3a9* expression[34]. Thus 1,25-D3 was unable to induce rat hepatic *Cyp3a1*, *Cyp3a2* or *Cyp3a9* mRNAs, but hepatic *Cyp3a9* induction was only achieved when liver slices were incubated with a higher concentration of LCA (50 μmol/L) and a longer duration (24 h)[34,35].

Furthermore, some other studies discussed the effects of other synthesized VDR ligands on intestinal *Cyp3a9* transcription, and the results differed from those of 1,25-D3 and LCA. 1α-Hydroxyvitamin D_2 (doxercalciferol), which was converted to the active form of 1,25-dihydroxyvitamin D_2 *in vivo*, was able to transactivate *Cyp3a9* in the rat liver and kidney, but not in the duodenum or ileum[37]. 19Nor-1,25-dihydroxyvitamin D_2, a VD_2 analog for the treatment of secondary hyperparathyroidism, interestingly showed a significantly stronger dose-dependent inductive effect on *Cyp3a9* mRNA than 1,25-D3 in rat intestine. The authors identified a proximal VDRE at the position −119 to −133 from the starting site of transcription in the antisense strand of *Cyp3a9* promoter. Another distal VDRE, at the position −727 to −783, was more responsive to 19nor-1,25-dihydroxyvitamin D_2 than 1,25-D3. The cell- and gene-specific regulation of activated VDR-RXR complex on different VDREs might result from the abilities of recruiting differentiated co-regulators. Therefore, the binding profiles of VD_2 analogs to VDR might be differentiated from that of 1,25-D3, and the ligand-VDR complex might selectively bind to different VDREs in VDR-target genes. In addition, the abilities of inducing CYP3As of VD_2 analogs may not correlate with disturbing intestinal calcium absorption and causing hypercalcemia, which were the usual side effects of 1,25-D3[1,107].

4.3.3. *Other rat CYP3A isoforms*

Although CYP3A18 and CYP3A62 served as main rat intestinal CYP3A isoforms[108], little is known about their induction. Modulation of these by PXR, CAR or VDR ligands has not been investigated.

Rats are much bigger than mice and easier to handle, which provide convenience for the more detailed study on the VDR-mediated CYP3A induction. The precise slices from different parts of the intestine (duodenum, jejunum, and ileum) and colon could be achieved, and the results discussed above indicated the differentiated expression of VDR and region-specific regulation on, for example, *Cyp3a1* and *Cyp3a2* mRNAs. The nonhomogeneous localization of CYP3A isoforms and VDR in different kinds of liver cells may also explain the isoform-specific induction after VDR ligand treatment. Nonetheless, many problems are still not solved as the results are sometimes inconsistent. For instance, there were controversial conclusions on the relative contributions of *Cyp3a1* and *Cyp3a9* genes to the total intestinal CYP3A protein. However, the enzymatic activity of rat intestinal homogenate (indicated as testosterone 6β-hydroxylation activity) was not changed. The authors hypothesized that, as the constitutive expression of CYP3A1 was much lower than CYP3A9 in intestinal epithelial cells, the increased CYP3A1 levels were not sufficient to elevate the catalytic activities[105]. In another study, total intestinal CYP3A protein levels were not changed after *in vivo* 1,25-D3 treatment, although *Cyp3a9* mRNA levels were increased by 2-fold and *Cyp3a1* mRNA levels remained unchanged[37]. As the antibodies to rat CYP3A were unable to distinguish between different isoforms, and the substrate reactions are not specific enough, the evaluation of the change in each CYP3A isoform was conducted more on transcriptional level than expressional or functional levels. Therefore, novel biochemical technologies and animal models are needed to help solve this problem and investigate more deeply into the interaction between VDR and rat CYP3As. Such work will also provide clues for the understanding of human CYP3A regulation and species discrepancies[109].

5. Conclusions

The up-regulatory effects of activated VDR on human CYP3A4 are clearly indicated in *in vitro* and *ex vivo* experiments, and the molecular mechanism is well elucidated. However, the clinical significance of related VDR polymorphisms and/or VDR-mediated CYP3A4 induction remains to be established. Mice and rats are two kinds of commonly used rodent animal models, in which the organ-specific and isoform-specific CYP3A induction by VDR ligands is also observed. It is noteworthy that most studies only focus on the mRNA level changes of CYP3As, but pay less attention to the changes of the relevant protein levels or metabolic activities, which might influence the pharmacokinetic profiles of CYP3A substrates. In particular, more *in vivo* evidence could be provided on VDR in the induction of CYP3A based on the *Vdr* KO mouse or rat models. Despite species discrepancies, the results from mice or rat experiments help to understand better the less focused pathway of CYP3A transcriptional regulation by VDR.

As compared with the broad spectrum of chemicals known to serve as the ligands for PXR and CAR, the ligands of VDR are previously restricted to VD_3 as well as its hydroxylated metabolites and the secondary bile acid LCA. Recently, the VDR-binding capacities of more compounds have been discovered, which show both nutritional and pharmacological significance. Although the affinities of these compounds towards VDR are much weaker than those of the natural ligands, these substances could be massively absorbed from foods and high local concentrations might be effective for VDR agonism. As VD_3 is an important nutrient and an endogenous chemical, the interplay between VD_3 and CYP3A4 should be a special focus, particularly under many pathological conditions and in chemotherapy.

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