Interplay between Vitamin D and the Drug Metabolizing Enzyme CYP3A4

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Abstract

Cytochrome P450 3A4 (CYP3A4) is a multifunctional enzyme involved in both xenobiotic and endobiotic metabolism. This review focuses on two aspects: regulation of CYP3A4 expression by vitamin D and metabolism of vitamin D by CYP3A4. Enterohepatic circulation of vitamin D metabolites and their conjugates will also be discussed. The interplay between vitamin D and CYP3A4 provides new insights into our understanding of how enzyme induction can contribute to vitamin D deficiency.

Keywords

Cytochrome P450 3A4; Vitamin D; Drug metabolism; Enterohepatic circulation; Osteomalacia

1. Vitamin D as a Hormone

Vitamin D consists of two isoforms, vitamin D₂ and vitamin D₃. Vitamin D₃ is the major source of the hormone in humans and is essential for the proper maintenance of calcium and phosphate homeostasis [1]. Once synthesized in skin after sunlight exposure, or absorbed in the gut from an oral diet, vitamin D₃ is delivered to the liver where it undergoes 25-hydroxylation by CYP2R1 and CYP27A1 (and other enzymes) to form 25-hydroxyvitamin D₃ (25OHD₃). Although 25OHD₃ is the major circulating form of vitamin D₃, it must be further oxidized at the 1α position by the enzyme CYP27B1 to 1α,25-dihydroxyvitamin D₃ [1α,25(OH)₂D₃] to become fully active at regulating gene transcription and cell function. 1α,25(OH)₂D₃ initiates or suppresses gene transcription by binding to the vitamin D receptor (VDR) [2]. Binding to VDR triggers hetero-dimerization of VDR with retinoid X receptor (RXR). The heterodimer then translocates to the nucleus where the complex binds to vitamin D response elements and alters gene transcription. In addition to this classical pathway of controlling cell function, several “non-genomic” pathways of cell regulation have also been proposed, involving extranuclear 1α,25(OH)₂D₃ and multiple growth factors [3].
CYP3A4 is considered to be the most important of the family of drug-metabolizing cytochrome P450 enzyme, contributing importantly to the clearance of perhaps half of therapeutic agents that undergo metabolic biotransformation [4]. It is highly expressed in the liver and small intestine [5] and several transcription factors interacting with multiple transcriptional elements regulate its expression. The induction of CYP3A4 gene expression in the liver and small intestine is mainly regulated through activation of the pregnane X receptor (PXR) and, in the case of the liver, also by the constitutive androstane receptor [6]. However, it has been demonstrated that 1α,25(OH)₂D₃ can also enhance the transcription of CYP3A4 by a VDR-mediated pathway. Treatment of Caco-2 cells [7–9], LS180 cells [10, 11], HepG2 cells [12], and human hepatocytes [13] with 1α,25(OH)₂D₃ results in increased CYP3A4 mRNA levels and accumulation of the encoded, functional enzyme. In addition, treatment of rats with 1α,25(OH)₂D₃ increases intestinal CYP3A23 (a homolog of CYP3A4) expression and CYP3A metabolic activity [14, 15].

CYP3A4 transcription is induced by 1α,25(OH)₂D₃ through the binding of ligand-VDR-RXR heterodimer to the same proximal ER6 (−169/−152), distal DR3 (−7733/−7719) and DR4 (−7618/−7603) response elements to which a ligand-PXR-RXR complex binds [16–18]. VDR also functions as a receptor for the secondary bile acid lithocholic acid (LCA) and binds (as an activated heterodimer) to the same CYP3A4 ER6 and DR3 response elements as does the 1α,25(OH)₂D₃-VDR-RXR complex [19]. Moreover, activation of VDR by LCA can induce CYP3A expression in vivo indicating that LCA may also contribute to CYP3A4 induction in the enteric system [20]. Finally, C-jun-N-terminal kinase has been suggested to be another mediator of 1α,25(OH)₂D₃-induced CYP3A4 expression, presumably without involvement of VDR [8, 21].

The primary sites of CYP3A4 expression are the liver and mucosa of the small intestine. Thus, it has been suggested that VDR helps regulate CYP3A4 enzyme content at these tissue sites [16, 22]. While this is clearly plausible for intestinal expression, it has been noted that the expression of VDR in human liver is much lower than that found in the small intestine and kidney [23]. Indeed, an immunohistochemical analysis of cross-sections of the human liver indicated that VDR is essentially absent from hepatocytes, but instead expressed in selective hepatic cell populations such as Kupffer, stellate and endothelial cells [24]. Therefore, considering the cellular composition of the liver, induction of hepatic CYP3A4 expression via VDR in non-parenchymal cells may not be that relevant to overall hepatic drug clearance [25], although additional work is needed to clarify this important regulatory issue.

Induction of CYP3A4 by 1α,25(OH)₂D₃ appears to affect the systemic exposure of orally administered drugs that are substrates of CYP3A4 (Figure 1). Humans taking vitamin D supplementation show an increased clearance of atorvastatin, a substrate of CYP3A4 [26]. In addition, blood levels of CYP3A substrates, tacrolimus and sirolimus, showed cyclic variation throughout the year that correlated with ultraviolet light exposure and serum levels of vitamin D, whereas no significant difference was observed for mycophenolic acid, a non-substrate of CYP3A4 [27]. Similarly, Thirumaran et al [25] reported that intestinal CYP3A4 expression in vivo varied seasonally, correlating with seasonal vitamin D levels. It is known that intestinal CYP3A4 contributes to the first-pass metabolism of many orally administered drugs [28]. Thus, intra- and inter-individual differences in circulating vitamin D levels and associated intestinal CYP3A4 activity may contribute to variability in oral drug bioavailability.
2. Enterohepatic Circulation of Vitamin D

Delivery of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ to the intestinal mucosa can in theory occur by both vascular and biliary routes [29]. In the absence of active uptake across the basolateral membrane of intestinal enterocytes, one would expect unbound intracellular $\alpha\text{,}25(\text{OH})_2\text{D}_3$ concentrations to be the same as that found in plasma, driving transcriptional activation of VDR gene targets such as $CYP3A4$, transient receptor potential cation channel, subfamily V, member 6 ($TRPV6$) and calbindin D9K. However, studies utilizing radiolabeled $\alpha\text{,}25(\text{OH})_2\text{D}_3$ in humans [30, 31] and animals [32] have revealed biliary excretion and intestinal reabsorption of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ (or its polar conjugate), suggesting the possibility of functionally significant enterohepatic cycling of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ (Figure 1). Although biliary vitamin D metabolites have not been fully characterized, chromatography indicates small amounts of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ and larger amounts of its more polar metabolites [31, 33]. Similar findings have been observed in man [34] and rats [35] after administration with radiolabeled 25OHD$_3$. A considerable portion of the radiolabeled $\alpha\text{,}25(\text{OH})_2\text{D}_3$ and 25OHD$_3$ dose found in the bile is subsequently absorbed from the intestine and presumably delivered back to the liver [32, 34]. Thus, although biliary excretion may not be an efficient pathway of vitamin D clearance, it may still expose enterocytes to higher unbound concentrations of active hormone (through vectoral transport through the enterocytes) than would otherwise occur by vascular delivery and represent an important element of gene regulation [34, 36].

A key initial step in enterohepatic cycling of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ and 25OHD$_3$ is conversion to a polar metabolite suitable for canalicular excretion. Glucuronidation of different vitamin D species has been reported [37–41]. In humans, the conjugation of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ is catalyzed primarily by UGT1A4 and to a much lesser extent by UGT2B4 and UGT2B7 [41]. Three isomers are produced, with the 25-O-glucuronide isomer being the dominant product. Delivery of 25-O-glucuronide of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ stimulates gene expression in mouse duodenum and colon tissues [42]. Glucuronides deposited in the gastrointestinal lumen are presumably subject to hydrolysis by $\beta$-glucuronidases (human epithelial or bacterial) after being secreted from the liver into the bile. Intraluminal release of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ and 25OHD$_3$ and subsequent reabsorption may serve as a critical tissue-specific hormone delivery pathway, giving explanation for a similar pattern of preferential expression of $CYP3A4$ [28], $TRPV6$ and calbindin D9K (Figure 2) in proximal sections of the small intestine, compared to more distal regions.

3. Biotransformation of Vitamin D by CYP3A4

The bioactivation of 25OHD$_3$ involves $\alpha$-hydroxylation, a reaction catalyzed primarily by CYP27B1 in the renal tubular epithelium [43]. The renal production of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ is tightly regulated by other hormones such as parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23), which up- and down-regulate expression of the $CYP27B1$ gene, respectively [44]. Furthermore, a variety of cell types in different organs of the human body contain the enzyme CYP27B1 and have the capacity to synthesize $\alpha\text{,}25(\text{OH})_2\text{D}_3$. It has been shown that local production of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ within nonrenal tissues is regulated in a manner different from that seen in the kidney, and thus, $\alpha\text{,}25(\text{OH})_2\text{D}_3$ production and action can be cell specific [45, 46]. Hydroxylation at the alkyl side chain of $\alpha\text{,}25(\text{OH})_2\text{D}_3$ or 25OHD$_3$ is considered to be a critical first step in the hormone inactivation pathway [47] (Figure 3). Mitochondrial CYP24A1 is recognized as a key enzyme for hydroxylation at the C-24 and C-23 positions of both 25OHD$_3$ and $\alpha\text{,}25(\text{OH})_2\text{D}_3$ [48, 49] and is found in kidney, intestine, lymphocytes, fibroblasts, bone, skin, macrophages and possibly other tissues where $\alpha\text{,}25(\text{OH})_2\text{D}_3$ exerts its biological effects [50]. However, Gupta et al [51, 52] reported that CYP3A4 exhibits significant 24- and 25-hydroxylation activities for $\alpha$-
OHD₃, 1α-OHD₂ and vitamin D₂, after screening 16 major human hepatic P450s expressed in baculovirus infected insect cells. Moreover, Xu et al [53] reported that CYP3A4 can catalyze the 23- and 24-hydroxylation of 1α,25(OH)₂D₃ in the human liver and small intestine. In addition, it was recently shown that CYP3A4 can catalyze 25OHD₃ monohydroxylation, generating nine different isomeric metabolites [54]. Interestingly, the major product generated by CYP3A4, 4β,25(OH)₂D₃, was detected in human plasma at concentrations comparable to that of 1α,25(OH)₂D₃ and its formation was induced by rifampin [54, 55]. Thus, intestinal and hepatic CYP3A4 may also contribute to the metabolic clearance of 1α,25(OH)₂D₃ and 25OHD₃, particularly under conditions of induced CYP3A4 expression.

4. Potential Clinical Implications

Osteomalacia can be a serious, debilitating side effect from certain drug therapies. It has been associated most strongly with chronic administration of anti-epileptic drugs, such as phenobarbital, carbamazepine, phenytoin and valproic acid [56–58], as well as the antimicrobial drug rifampin [59, 60]. Although the molecular mechanism underlying this event is not fully understood, a number of theories have been proposed to explain why anti-epileptic drugs affect bone; this includes reduced levels of vitamin D metabolites, reduced calcium absorption, inhibition of the cellular response to PTH, hyperparathyroidism, vitamin K deficiency and calcitonin deficiency [61]. Among them, vitamin D deficiency is thought to be the principal pathway to drug-induced osteomalacia [62, 63]. For example, daily administration of rifampin to healthy volunteers lowered the plasma concentration of 25OHD₃ by 70% [60, 64]. Similar effects were also described for patients receiving phenobarbital and phenytoin for the treatment of epilepsy [65]. It has been suggested that alterations in vitamin D metabolism result mainly from induction of hepatic P450 enzymes [66, 67]. Pascussi et al [68] proposed that induction of CYP24A1 accelerates vitamin D catabolism, and that PXR is responsible for the regulation of CYP24A1 enzyme. In contrast, Zhou et al [69] reported that CYP3A4, and not CYP24A1, is the dominant enzyme catalyzing hydroxylation of 1α,25(OH)₂D₃ in human liver and small intestine. Thus, the relative contribution of these two enzymes to vitamin D catabolism may be tissue-specific. In the healthy kidney, CYP24A1 activity likely dominates 1α,25(OH)₂D₃ catabolism; while in the liver and small intestine, CYP3A4 activity most likely dominates vitamin D catabolism because of a much greater level of basal and induced enzyme expression [69]. This interpretation is supported by the observation that treatment of healthy volunteers with rifampin causes preferential increases in human duodenal CYP3A4, but not CYP24A1, mRNA content [69]. Importantly, induction of CYP3A4 in the small intestine could cause local tissue vitamin D deficiency and possibly directly affect intestinal calcium absorption [11, 70]. On the other hand, induction of hepatic CYP3A4 may alter systemic circulating levels of 25OHD₃ through activation of the 4β-hydroxylation pathway [54]. Therefore, inhibition of CYP3A4 activity in the enterohepatic circuit may constitute a viable therapeutic approach for prevention or reversal of drug-induced osteomalacia in at-risk patients.

Liver function is crucial for physiological vitamin D metabolism. Patients with chronic liver disease and cirrhosis have very high prevalence of vitamin D deficiency and insufficiency [71]. Low vitamin D levels in these patients are possibly due to limited sunlight exposure, impaired intestinal absorption and decreased hepatic 25-hydroxylation activity [71]. Given the correlation between plasma levels of vitamin D metabolites and intestinal CYP3A4 content/activity, low levels of vitamin D metabolites in patients with cirrhosis might result in a reduction in CYP3A protein expression. Indeed, it has been noted that intestinal CYP3A4 expression and function are reduced in patients with cirrhosis [72]. Although reduced metabolism of drugs is typically attributed to decreased liver function, decreased
intestinal CYP3A activity may also contribute to greater drug exposure in these patients. Whether vitamin D supplementation could rescue intestinal CYP3A expression in these patients is something to be considered.

5. Concluding remarks

Hormonal control of CYP3A4 expression by vitamin D represents the foundation of a potentially important interplay between xenobiotic and vitamin D metabolism. Enterohepatic cycling of vitamin D could be a functionally important pathway for delivery of active hormone to the upper intestine, resulting in preferentially higher levels of expression of VDR target genes, such as TRPV6, calbindin D9K and CYP3A4, in the duodenum and jejunum, in comparison to the ileum and colon. Intra- and inter-individual differences in vitamin D levels may contribute to the considerable variability in intestinal CYP3A4 content that affects drug disposition and pharmacological response. Interestingly, CYP3A4 catalyzes vitamin D biotransformation down pathways that appear catabolic in nature. Certain drugs, such as anti-epileptic drugs, that can induce CYP3A4 expression in the liver and small intestine, accelerate vitamin D catabolism and may contribute to vitamin D deficiency, although a causal mechanistic link between CYP3A4 induction and vitamin D deficiency requires further evaluation.

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References


Highlights

1. Regulation of CYP3A4 gene expression by vitamin D
2. Enterohepatic circulation of vitamin D metabolites
3. Metabolism of vitamin D by CYP3A4
4. Vitamin D deficiency and clinical implications
Figure 1.
Proposed mechanism that enterohepatic circulation delivers vitamin D derivatives to the intestine and 1α,25(OH)₂D₃ induces intestinal CYP3A4 expression via VDR-mediated pathway.
Figure 2. Longitudinal distribution of TRPV6 and calbindin D9k mRNA in the human small intestine

Tissue samples from four different donors were presented. Isolation of total RNA and real-time quantitative PCR were performed as previously described [53]. A) the levels of TRPV6 mRNA were normalized to the corresponding GAPDH mRNA; B) the levels of Calbindin D9K mRNA were normalized to villin mRNA.
Figure 3. Tissue-specific metabolism of vitamin D metabolites
Under the constitutive condition in the kidney, CYP27B1 catalyzes 1α-hydroxylation of 25OHD$_3$ for vitamin D activation. While CYP24A1 catalyzes 24R- and 23S-hydroxylation of 25OHD$_3$ and 1α,25(OH)$_2$D$_3$ for vitamin D deactivation. In the liver, CYP3A4 is the most abundant enzyme that catalyzes 4β-hydroxylation of 25OHD$_3$, and 23R- and 24S-hydroxylation of 1α,25(OH)$_2$D$_3$ for vitamin D deactivation.