Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 360 (2007) 76–82
BBRC
www.elsevier.com/locate/ybbrc

Constitutive androstane receptor–vitamin D receptor crosstalk: Consequence on *CYP24* gene expression

Amélie Moreau, Patrick Maurel, Marie-José Vilarem, Jean-Marc Pascussi *

Inserm, Université Montpellier1, UMR-632, Montpellier F-34293, France

Received 23 May 2007
Available online 13 June 2007

Abstract

We previously reported that the pregnane X receptor (PXR) interferes with vitamin D receptor (VDR) target genes, notably *CYP24*, by targeting the same responsive elements. Since PXR and constitutive androstane receptor (CAR) share responsive elements in the promoter of their target genes, we wondered whether CAR also interferes with CYP24 expression. The current study shows that: (i) CAR-RXR heterodimer binds to and transactivates the proximal promoter of *CYP24* (−1200/+22) and both VDRE-1 and VDRE-2 which control its expression in response to 1,25-dihydroxyvitamin D_3, (ii) androstanol an inverse agonist of hCAR inhibits transactivation of VDREs by hCAR, (iii) mutations of either VDRE-1 or -2 half sites inhibit hCAR-mediated transactivation, and (iv) in primary human hepatocytes ($n = 11$) CITCO, a specific hCAR agonist, is an inducer of CYP24 as well as of CYP2B6 and CYP3A4 mRNAs. In conclusion, CAR/PXR and VDR bind to and transactivate the same response elements in *CYP24* promoter.

Keywords: CAR; VDR; PXR; Crosstalk; Vitamin D3; CYP24; Osteomalacia

Vitamin D receptor (VDR, NR1I1), a member of the non-steroid receptor superfamily, controls a large battery of genes mediating biological functions such as cell differentiation, apoptosis, immunomodulation, calcium and phosphate homeostasis, and bone mineralisation [1]. Its major agonist is the 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2$-D) which results from hydroxylation of vitamin D at position 25 and 1 in liver and kidney, respectively, by appropriate CYP enzymes [2,3]. After ligand binding, VDR forms a heterodimer with RXRα which transactivates the vitamin D response elements (VDRE) present in the regulatory region of target genes. The consensus sequence of VDREs is a direct repeat of the core AGGTCA separated by three nucleotides (DR3). One of the VDR target genes is *CYP24* which encodes a mitochondrial cytochrome P450 that hydroxylates $1,25(OH)_2$-D and other vitamin D derivatives at position 24 [4,5]. This biotransformation converts these molecules to inactive metabolites. *CYP24* expression is dependent on two responsive elements (VDRE-1 and VDRE-2) located in the proximal region of the promoter [6,7]. Transactivation of these elements by VDR/RXR leads to increased expression of CYP24, reduced level of the biologically active $1,25(OH)_2$-D, and downregulation of VDR-target genes. Vitamin D homeostasis appears therefore to be autoregulated at least in part through VDR and CYP24.

The NR1I family comprises at least two other members, the pregnane X receptor (PXR, NRI12) and the constitutive androstane receptor (CAR, NR1I3). Both PXR and CAR form heterodimers with RXR and bind to and transactivate various response elements (including ER6, DR3, and DR4) present in the promoter of their target genes [8,9]. PXR and CAR thus control the inducible expression of a wide battery of genes involved in xenobiotic and drug metabolism and transport including *CYP2* and *CYP3* families, *UGTs*, *STs*, *MDRs*, *MRP*, etc [10]. Several groups have reported that, when activated by $1,25(OH)_2$-D, VDR is able to induce *CYP2* and *CYP3* gene expression in different cell types including primary human hepato-

* Corresponding author.
E-mail address: pascussi@montp.inserm.fr (J.-M. Pascussi).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.003

cytes, and this was shown to result from the fact that VDR binds to and transactivates the PXR–CAR responsive elements present in the promoter of these genes [11–13]. More recently, we reported that PXR binds to and transactivates VDRE-1 and VDRE-2 of the *CYP24*, and that PXR agonists increase CYP24 mRNA expression in human hepatocytes and mouse kidney [14]. These findings suggest that the VDR–PXR crosstalk resulting from the recognition of same response elements is reciprocal. Such crosstalk provides, at least in part, an objective explanation to the observation that long-term treatment of patients with drugs that are PXR agonists results in low $1,25(OH)_2$-D levels and osteomalacia [15–17].

Since PXR and CAR have been reported to share responsive elements in the promoter of their target genes [18–22], we decided to determine whether CAR interferes with VDR target genes as well. The data presented here show that CAR binds to and transactivates the VDREs present in the *CYP24* promoter, and that CITCO, a specific human CAR agonist [23], increases CYP24 mRNA expression in primary human hepatocytes.

Materials and methods

Chemicals. Culture media, dimethylsulfoxide, rifampicin, 1α,25-dihydroxyvitamin D_3 ($1,25-(OH)_2D$), and culture medium additives were from Sigma (Saint Louis, MO). γ-[^{32}P]dATP was from Amersham International (Amersham, England). SR12813 were purchased from Tebu-bio (Le Perray en Yvelines, France).

Plasmids. The following plasmids have been described previously: pSG5-Δ^{ATG}-hPXR, pcDNA3.1-his-tagged PXR, pSG5-mPXR, pSG5-mRXRα, pSG5-hVDR, pGL3-hCYP24 (−1200/22)-LUC, p(NR1)3-tkLUC, p(VDRE-1)3-tkLUC, and p(VDRE-2)3-tkLUC [11,14]. The hCAR expression vectors were generated by PCR, using pCDM8-hCAR vector as a template (kindly provided by M. Negishi) and oligonucleotides sense hCAR/ATG 5′-CGGAATTCATGGCCAGTAGGGAAG and reverse hCAR/TGA 5′-AAAAAAGCGGCCGCCTCAGCTGCAGATC TCCTGG and cloned into plasmids pcDNA3 (Invitrogen, Groningen, The Netherlands). All subcloned fragments including CYP2B6 NR1 and CYP24 VDRE-1 and -2 were verified by sequencing.

Primary culture of human hepatocytes. Hepatocytes were prepared and cultured according to the previously published procedure [24]. Clinical characteristics of livers and lobectomy donors used in this work are summarized in Table 1.

Quantitative PCR. Total RNA was extracted using TRIZOL reagent (Gibco-BRL, Cergy-Pontoise, France). Purity was confirmed by spectrophotometer. cDNA were synthesized from 1 μg of total RNA using Superscript II first-strand synthesis system for PCR (Invitrogen) at 42 °C for 60 min, in the presence of random hexamers (Amersham-Pharmacia Biotech). Quantification of CYP24, CYP2B6, CYP3A4, GAPDH, and β-actin mRNA was performed using the Roche Light Cycler apparatus as described. Sense and reverse primers were as follows, respectively:

GAPDH (NM_002046), 5′-GGTCGGAGTCAACGGATTTGGTCG and 5′-CAAAGTTGTCATGGATGACC; β-actin (NM_001101), 5′-TG GGCATGGGTCAGAAGGAT and 5′-TCCATCACGATGCCAGTG GT; CYP2B6 (NM_000767), 5′-GGCCATACGGGAGGCCCTTG and 5′-AGGGCCCCTTGGATTTCCG; CYP24 (CYP24A1, NM_000782): 5′-GTGGCTCCAGCCAGACCCTA and 5′-GGCGAGGTTGGTACGAG GTG, CYP3A4 (NM_017460): 5′-CACAAACCGGAGGCCTTTTG and 5′-ATCCATGCTGTAGGCCCCAA. CYP24 mRNAs amplified fragments were purified using the Nucleospin extract kit (Macherey–Nagel) and verified by sequencing.

Cell culture and transfections. Hek293 cells (human embryonic kidney cells) were cultured and transfected as reported [14]. As internal control, 50 ng of pSV-β-galactosidase (Promega) was added. After 12–16 h, the medium was changed, and fresh medium containing 5% delipidated-charcoal stripped serum (Sigma) and 0.1% DMSO (vehicle) or inducers was added. Twenty-four hours after changing media, the cells were harvested in reporter lysis buffer (Promega) and cells extracts were analysed for luciferase and β-galactosidase activities as described. Luciferase values were normalized with β-galactosidase values calculated by dividing the maximal response by the response elicited with vehicle.

Electromobility shift assays. Double-stranded oligonucleotides corresponding to the two VDREs of the human *CYP24* gene were synthetized (Invitrogen): VDRE-1 (–174 to –151): CCGGACGCCCTCGCTCA CCTCGCTGA; VDRE-2 (−294 to –274): CGAAGCACACCC GGTG AACTCCGG. CYP2B6 NR1 probe and binding procedure were previously described [11,14]. Probes were end-labeled with [^{32}P]-ATP using T4 polynucleotide kinase (Promega), and then incubated 20 min at room temperature with 1 μL of *in vitro* synthetized CAR, PXR, his-tagged PXR, VDR or RXRα (TNT-coupled transcriptional translation, Promega). DNA–protein complexes were resolved on a 4% polyacrylamide gel (30:1, acrylamide:bis-acrylamide) in 0.5× TBE (1× TBE = 89 mM Tris, 89 mM boric acid, and 2 mM EDTA). Gels were dried and subjected to autoradiography with Kodak X-AR film.

Results

CAR binds to and transactivates VDRE-1 and VDRE-2 of CYP24 promoter

Analysis of VDR- and CAR-binding to specific response elements has been assessed by electromobility shift assay. Radiolabeled *CYP2B6* NR1 (a typical target of CAR/RXR) and *CYP24* VDRE-1 and VDRE-2 oligonucleotides were incubated in the absence or presence of CAR, VDR, and RXRα proteins prepared by a transcription–translation coupled system, either alone or in association, before being loaded onto the gel. PXR and his-tagged PXR proteins were also included in these series of experiments as control of our previous observations [14]. The data of Fig. 1 (left) shows that the heterodimer CAR/RXR binds strongly to *CYP2B6* NR1, as expected [25]. Both VDR/RXR and PXR/RXR heterodimers also bind this oligonucleotide albeit with an apparently lower affinity. This is consistent with the well established fact that CAR and PXR share response elements in their target genes [18–22], and that $1,25(OH)_2$-D is a transcriptional inducer of CYP2B6 and CYP3A4 [11–13]. Note that the mobility of the VDR/RXR complex is slightly lower than that of the CAR/RXR complex and slightly faster than that of the PXR/RXR complex. The data shown in Fig. 1 (right) reveals that the CAR/RXR heterodimer binds to both VDRE-1 and VDRE-2 of *CYP24* promoter. As expected from previous observations [14], both VDR/RXR and PXR/RXR heterodimers bind these response elements as well although the affinity for VDR/RXR seems greater. These results show that the CAR/RXR heterodimer binds to *CYP24* VDRE-1 and VDRE-2, as does the PXR/RXR heterodimer.

In a next series of experiments, the capacity of CAR to transactivate both *CYP24* VDREs was investigated by cotransfection assays. For this purpose, HEK293 cells derived from human embryonic kidney were tran-

Table 1
Clinical characteristics of livers and lobectomy donors

Donor identification	Gender	Age	Pathology
FT 228	M	76	Metastasis of colon cancer
FT 237	F	78	Metastasis of colon cancer
FT 256	F	43	Adenoma on normal liver
FT 257	F	30	Adenoma on normal liver
FT 258	F	76	Metastasis of colon cancer
FT 259	M	50	Adenoma on normal liver
FT 261	F	21	Adenoma on normal liver
FT 263	F	79	Hepatocarcinoma
FT 264	M	68	Metastasis of colon cancer
FT 266	F	70	Adenoma on normal liver
FT 267	F	55	Metastasis of colon cancer
FT 268	F	48	Metastasis of colon cancer
FT 269	F	46	Adenoma on normal liver
FT 270	M	57	Metastasis of colon cancer
FT 271	F	56	Metastasis of colon cancer
FT 272	F	24	Donor
FT 273	M	69	Metastasis of colon cancer
FT 275	M	73	Metastasis of bladder cancer
FT 276	F	62	Metastasis of pancreas cancer
FT 277	M	48	Mucin cystadenoma
FT 278	M	71	Metastasis of rectum cancer
FT 279	F	57	Metastasis of breast cancer
FT 280	M	59	Unknown
FT 281	F	56	Metastasis of colon cancer

siently transfected with a human CAR expression vector (pSG5-hCAR) or the control expression vector (pSG5), in the presence of reporter plasmids pGL3-hCYP24 (−1200/22)-LUC, p(VDRE-1)3-tkLUC or p(VDRE-2)3-tkLUC (including wild-type and mutated VDRE-1 and -2). Human PXR (pSG5-Δ^{ATG}-hPXR) and VDR (pSG5-hVDR) expression vectors were tested in parallel experiments. Cells were then treated for 24 h with vehicle (0.1% DMSO; UT), 10 μM androstanol (Anol, a well-known human CAR inverse agonist) [26], 50 nM 1,25(OH)$_2$-D (VD) or 10 μM rifampicin (RIF). The data on reporter gene activity is reported in Fig. 2. In the absence of activator, transfection of CAR produced a potent induction of the reporter gene, whatever the driving region, the −1200/+22 promoter of human *CYP24* or the *CYP24* VDRE sequences (Fig. 2A and B), and incubation of cells with androstanol decreased this induction. This is consistent with the fact that CAR exhibits a strong constitutive transcriptional activity [25] and that androstanol is a well-known inverse agonist of this receptor [26]. Mutation of either of the VDREs strongly reduced the reporter gene activity suggesting that transactivation of these elements by CAR requires the wild type sequence (Fig. 2C and D). In parallel experiments, VDR and PXR transactivated the same reporter constructs in the presence of 50 nM 1,25(OH)$_2$-D or 10 μM rifampicin, respectively, as expected. Here again, mutation of both VDREs strongly reduced the reporter gene activity. Collectively, these results show that CAR binds to and transactivates both VDREs of the *CYP24* promoter.

CITCO and other CAR and PXR activators induce the expression of CYP24 mRNA in primary human hepatocytes

In order to determine whether CAR activation results in increased expression of *CYP24* gene in a more physiologically pertinent experimental model, human hepatocytes in primary culture were treated for 48 h with CITCO, a specific agonist of human CAR [23], and CYP24, CYP2B6 and CYP3A4 mRNA expression was assessed by quantitative RT-PCR. Note that in these experiments, CITCO was used at 100 nM, a concentration at which PXR is not activated; indeed, we have observed that concentrations greater than 1 μM activate PXR. In parallel experiments, cells were treated with vehicle (0.1% DMSO; UT), 0.5 mM phenobarbital (a mixed activator of CAR and PXR), 20 μM rifampicin or 1 μM SR12823 (two potent agonists of PXR). The data reported in Table 2 was collected from the analysis of different primary hepatocyte cultures prepared from different donors (see Table 1). It is of note that there were large interindividual variations in the extent to which *CYP24* and other *CYP* gene expression was increased in response to the various compounds. This accounts for the large standard deviations observed in some cases. The data shows that CITCO is a significant inducer ($p < 0.05$, $n = 11$) of CYP24, CYP2B6, and CYP3A4 mRNAs with similar fold-induction ratios. This is consistent with the results presented in Figs. 1 and 2 showing that CAR binds to and transactivates both *CYP24* VDREs. However, CITCO was a weaker inducer of the three tested genes, especially as compared with rifampicin and SR12823. This suggests that PXR is a more potent transactivator of *CYP24* as compared with CAR. The fold-induction ratio of CYP24 mRNA is approximately one order of magnitude smaller than that characterising CYP3A4 in response to phenobarbital, rifampicin and SR12823, while the fold-induction ratio of CYP2B6 appears to be intermediate. Hence, although CYP24 mRNA is indeed induced by both CAR and PXR agonists, the amplitude of the increase is significantly smaller than that of CYP2B6 and CYP3A4 mRNAs. This is the mirror image of what was observed on the induction of these genes in response to 1,25(OH)$_2$-D [11,14].

Discussion

The results reported here show that: (i) CAR/RXR heterodimer binds to and transactivates the proximal promoter of *CYP24* (−1200/+20) and both VDRE-1 and VDRE-2 which control the expression of *CYP24* in response to 1,25(OH)$_2$-D, (ii) androstanol a well-characterised inverse agonist of hCAR inhibits the transactivation of VDREs by hCAR, (iii) mutations of either VDRE-1 or -2 half sites inhibit hCAR-mediated transactivation, and (iv) in primary human hepatocytes CITCO, a specific CAR agonist, is an inducer of CYP24 as well as of CYP2B6 and CYP3A4 mRNAs.

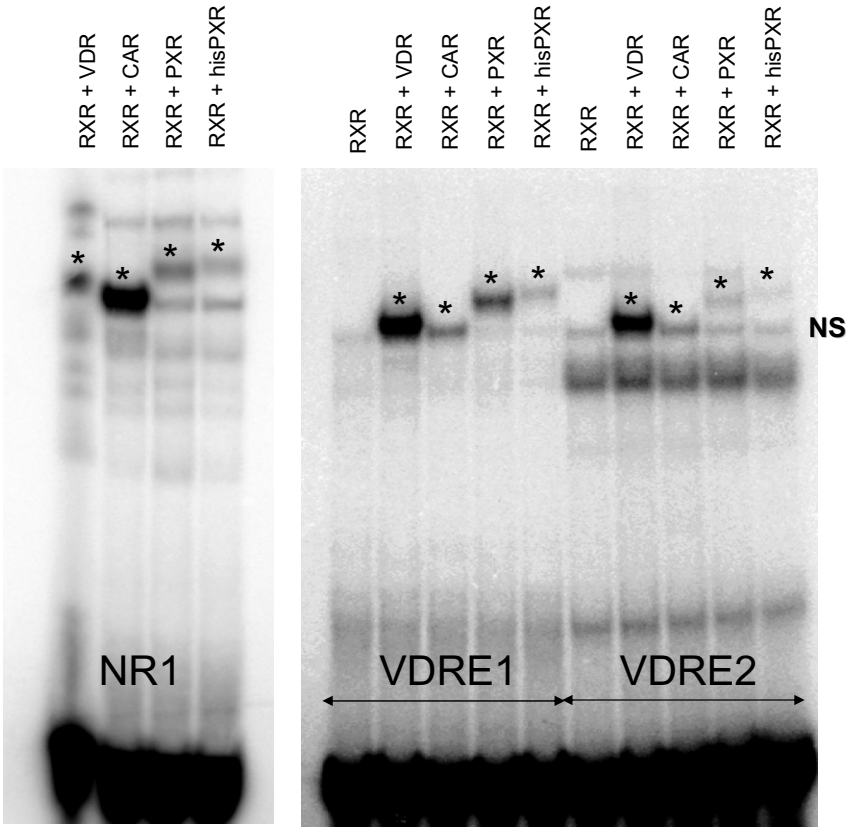

Fig. 1. Analysis of VDR-, CAR- and PXR-binding to specific response elements by electromobility shift assay. Radiolabeled CYP2B6 NR1 and CYP24 VDRE-1 and VDRE-2 oligonucleotides (50,000 cpm) were incubated in the absence or presence of VDR, CAR, PXR, his-tagged PXR, and RXRα proteins prepared by *in vitro* translation using a transcription–translation coupled system, either alone or in association as indicated, before being loaded onto the gel. Complexes are identified by a start (*) just above the band. NS refers to nonspecific band. Left, binding to CYP2B6 NR1 oligomer. Right, binding to CYP24 VDRE-1 and VDRE-2 oligomers.

The recognition of specific response elements by nuclear receptors in the promoter of their target genes is a major determinant of the control of many physiological functions by appropriate stimuli including hormones, xenobiotics and others [27]. Many reports, however, have revealed that the specificity exhibited by nuclear receptors for their response elements is not as high as first thought; indeed, some receptors bind to and transactivate DNA motifs that are significantly different from their so-called "consensus" response element. This has been shown to be true for several nuclear receptors such as the glucocorticoid receptor, estrogen receptor, androgen receptor, VDR, CAR and PXR [18,19,22,28–32]. It is therefore not surprising that CAR is able to target the DR3-type response elements of *CYP24* promoter, while this receptor targets DR3, DR4, and ER6 elements in *CYP2B6*, *CYP2C9,* and *CYP3A4* gene promoters. The apparent ability of a given nuclear receptor to target distinct DNA sequences is believed to result from the flexibility of either the DNA-binding domain or the hinge domain or from the association with other transcription factors or the promoter context. From a functional point of view, this versatility leads to the possibility of crosstalk such as the one investigated here.

The observation of osteomalacia and bone mineralisation disorders in patients treated with agonists or activators of PXR and CAR has been the incentive of this work. The present finding that CAR, in addition to PXR [14], might increase the expression of *CYP24* provides a further objective molecular mechanism by which some xenobiotics affect $1,25(OH)_2$-D level and bone mineralisation in man under prolonged therapy [15–17].

Interestingly, it was reported recently that CYP2D25, the porcine microsomal vitamin D 25-hydroxylase, is downregulated at the transcriptional level by VDR in the presence of vitamin D metabolites and by both CAR and PXR in the presence of phenobarbital [33,34]. These observations are in favor of another aspect of the crosstalk between CAR, PXR and VDR. However, induction of CYP24 by xenobiotics *via* CAR or PXR is certainly not the only molecular mechanism by which xenobiotics might induce bone disorders. Indeed, Xu et al. [35] demonstrated that, in addition to CYP24, CYP3A4 may efficiently contribute to the catabolism of $1,25(OH)_2$-D and other vitamin D metabolites by generating 23*R*- and 24*S*-hydroxy metabolites in liver and intestine under xenobiotic treatment.

Finally, Zhou et al. [36] reporting on the PXR–VDR crosstalk recently concluded, in disagreement with our

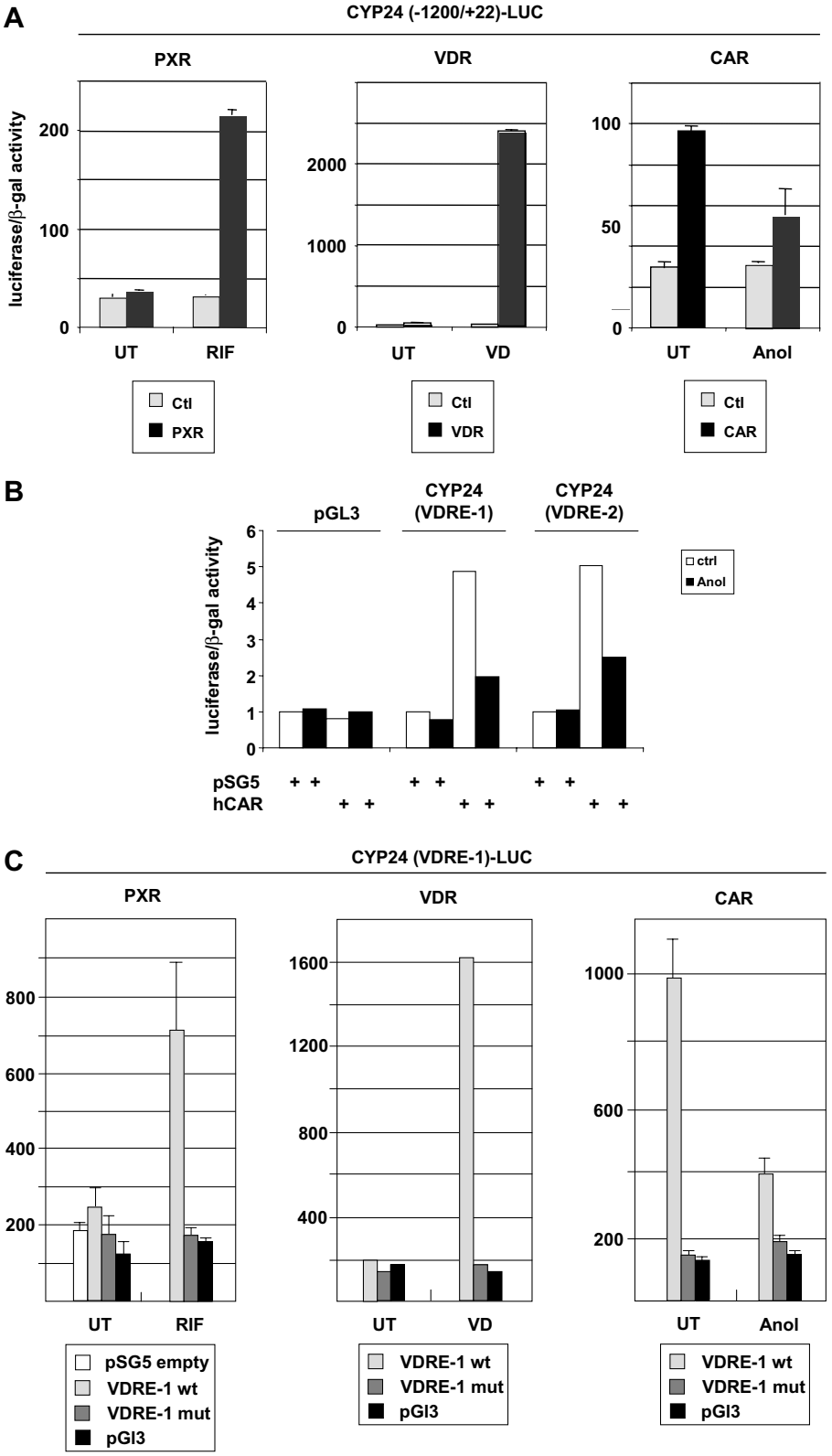

Fig. 2. Transactivation of CYP24 promoter by VDR, CAR, and PXR. HEK293 cells (human kidney) were transiently transfected with human VDR (pSG5-hVDR), CAR (pcDNA3.1-hCAR) or PXR (pSG5-Δ^{ATG}-hPXR) or control expression vector (pSG5, Ctl), in the presence of reporter vector (pGl3-luciferase) driven by the indicated human CYP24 promoter sequences, together with pRSV-β-gal transfection control plasmid. Cells were treated with vehicle (0.1% DMSO; UT), 50 nM 1,25$(OH)_2$D3 (VD), 10 μM rifampicin (RIF) or 10 μM androstanol (Anol) for 24 h. Cells were then harvested and analyzed for both luciferase and β-galactosidase activities. Values represent β-galactosidase-normalised luciferase activities and are the average of duplicates ± SE. These were replicated in independent experiments. (A) CYP24 promoter region −1200/+22. (B) CYP24 VDRE-1 and -2 transactivation by CAR. (C) CYP24 VDRE-1 wild-type or mutated site. (D) CYP24 VDRE-2 wild-type or mutated site.

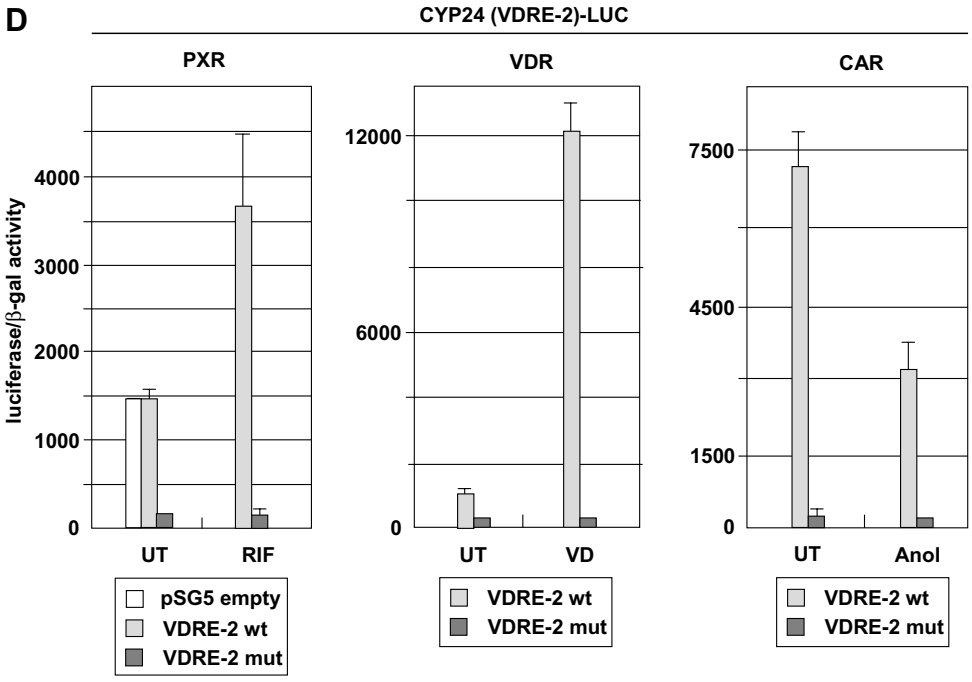

Fig 2. *(continued)*

Table 2
Fold-induction of CYP24, CYP2B6, and CYP3A4 mRNA by various inducers in cultured human hepatocytes

Inducers[a]	CYP24[b]	CYP2B6 [b]	CYP3A4 [b]
CITCO	1.8 ± 1.2 ($n = 12$) (0.9–5.2) $p = 0.02$	2.5 ± 1.95 ($n = 11$) (0.7–7.5) $p = 0.016$	3.5 ± 3 ($n = 12$) (0.9–12.4) $p = 0.009$
Phenobarbital	4.0 ± 1.7 ($n = 5$) (1.6–6.1) $p = 0.009$	10.7 ± 9.9 ($n = 12$) (1.4–31.7) $p = 0.003$	87.2 ± 190.6 ($n = 13$) (1.9–705) $p = 0.0664$
Rifampicin	8.0 ± 6.2 ($n = 10$) (0.9–20) $p = 0.003$	13.7 ± 10.6 ($n = 15$) (2–31.2) $p = 0.002$	84.2 ± 82.2 ($n = 16$) (1.1–278) $p = 0.0005$
SR	3.37 ± 2.58 ($n = 8$) (0.2–8.6) $p = 0.02$	27.5 ± 44.6 ($n = 8$) (2.8–135) $p = 0.07$	65.06 ± 77.11 ($n = 8$) (10.6–225) $p = 0.03$

[a] Human hepatocytes were cultured in our standard conditions (ISOM) in the absence or presence of indicated inducers for 48 hours. CYP24, CYP2B6, CYP3A4 mRNA levels were evaluated by quantitative RT-PCR as described [1] using sequence-checked primers, and normalised to the levels of β-actin mRNA; β-actin mRNA levels were insensitive to the various inducers used. Induction ratios (mRNA in inducer-treated cells/mRNA in untreated cells) ± standard error are presented here. RIF, rifampicin (20 μM); SR, SR128123 (1 μM); PB, phenobarbital (500 μM); CITCO (100 nM).

[b] *n*, number of different hepatocyte cultures tested (prepared from different patients); numbers in parentheses refer to the range of variation; *p*, Student's *t*-test.

previous observations [14], that CYP24 is not induced but rather downregulated by PXR agonists. In particular, these authors observed no binding to nor transactivation of the *CYP24* promoter by PXR, nor did they observed any induction of CYP24 mRNA by PXR agonists in primary human hepatocytes. However, during the course of the present work, some of the experiments that were carried out in our previous investigation [14] had to be repeated as necessary controls. These included PXR binding to and transactivation of *CYP24* promoter and VDREs, and induction of CYP24 mRNA in primary human hepatocytes. All these experiments (reported in Figs. 1 and 2 and Table 2) fully confirmed our previous findings on the PXR–VDR crosstalk. Importantly, all human hepatocyte cultures ($n = 11$ or 12 for CYP24 induction by CITCO or rifampicin, respectively) used in the present work were posterior to the date of submission of our previous paper [14], pointing to the reproducibility of experiments with different hepatocyte cultures. We therefore, believe that the discrepancies between our work and that published by Zhou et al. [36] are due to methodological differences in hepatocyte cultures, transfection assays (nature of cell lines) and electromobility shift assays.

In conclusion, CAR, PXR, and VDR appear to be able to bind to and transactivate the same response elements, notably in the *CYP24* promoter. This is likely to explain, at least in part, the xenobiotic-induced bone disorders in patients under prolonged therapy with drugs that are activator/agonist of CAR or PXR.

References

[1] C. Carlberg, Current understanding of the function of the nuclear vitamin D receptor in response to its natural and synthetic ligands, Recent Results Cancer Res. 164 (2003) 29–42.

[2] A.S. Dusso, A.J. Brown, E. Slatopolsky, Vitamin D, Am. J. Physiol. Renal. Physiol. 289 (2005) F8–F28.

[3] K. Wikvall, Cytochrome P450 enzymes in the bioactivation of vitamin D to its hormonal form (review), Int. J. Mol. Med. 7 (2001) 201–209.

[4] B. Endres, H.F. DeLuca, 26-Hydroxylation of 1alpha,25-dihydroxyvitamin D3 does not occur under physiological conditions, Arch. Biochem. Biophys. 388 (2001) 127–134.

[5] A.L. Sutton, P.N. MacDonald, Vitamin D: more than a ''bone-a-fide'' hormone, Mol. Endocrinol. 17 (2003) 777–791.

[6] K.S. Chen, H.F. DeLuca, Cloning of the human 1 alpha,25-dihydroxyvitamin D-3 24-hydroxylase gene promoter and identification of two vitamin D-responsive elements, Biochim. Biophys. Acta 1263 (1995) 1–9.

[7] D.M. Kerry, P.P. Dwivedi, C.N. Hahn, H.A. Morris, J.L. Omdahl, B.K. May, Transcriptional synergism between vitamin D-responsive elements in the rat 25-hydroxyvitamin D3 24-hydroxylase (CYP24) promoter, J. Biol. Chem. 271 (1996) 29715–29721.

[8] L.A. Stanley, B.C. Horsburgh, J. Ross, N. Scheer, C.R. Wolf, PXR and CAR: nuclear receptors which play a pivotal role in drug disposition and chemical toxicity, Drug Metab. Rev. 38 (2006) 515–597.

[9] Y.E. Timsit, M. Negishi, CAR and PXR: the xenobiotic-sensing receptors, Steroids 72 (2007) 231–246.

[10] J.J. Eloranta, P.J. Meier, G.A. Kullak-Ublick, Coordinate transcriptional regulation of transport and metabolism, Methods Enzymol. 400 (2005) 511–530.

[11] L. Drocourt, J.C. Ourlin, J.M. Pascussi, P. Maurel, M.J. Vilarem, Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes, J. Biol. Chem. 277 (2002) 25125–25132.

[12] P. Schmiedlin-Ren, K.E. Thummel, J.M. Fisher, M.F. Paine, P.B. Watkins, Induction of CYP3A4 by 1 alpha,25-dihydroxyvitamin D3 is human cell line-specific and is unlikely to involve pregnane X receptor, Drug Metab. Dispos. 29 (2001) 1446–1453.

[13] K.E. Thummel, C. Brimer, K. Yasuda, J. Thottassery, T. Senn, Y. Lin, H. Ishizuka, E. Kharasch, J. Schuetz, E. Schuetz, Transcriptional control of intestinal cytochrome P-4503A by 1alpha,25-dihydroxy vitamin D3, Mol. Pharmacol. 60 (2001) 1399–1406.

[14] J.M. Pascussi, A. Robert, M. Nguyen, O. Walrant-Debray, M. Garabedian, P. Martin, T. Pineau, J. Saric, F. Navarro, P. Maurel, M.J. Vilarem, Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia, J. Clin. Invest. 115 (2005) 177–186.

[15] D.L. Andress, J. Ozuna, D. Tirschwell, L. Grande, M. Johnson, A.F. Jacobson, W. Spain, Antiepileptic drug-induced bone loss in young male patients who have seizures, Arch. Neurol. 59 (2002) 781–786.

[16] T.Y. Chan, Osteomalacia during rifampicin and isoniazid therapy is rare in Hong Kong, Int. J. Clin. Pharmacol. Ther. 34 (1996) 533–534.

[17] Y. Karaaslan, S. Haznedaroglu, M. Ozturk, Osteomalacia associated with carbamazepine/valproate, Ann. Pharmacother. 34 (2000) 264–265.

[18] S. Gerbal-Chaloin, M. Daujat, J.M. Pascussi, L. Pichard-Garcia, M.J. Vilarem, P. Maurel, Transcriptional regulation of CYP2C9 gene. Role of glucocorticoid receptor and constitutive androstane receptor, J. Biol. Chem. 277 (2002) 209–217.

[19] B. Goodwin, E. Hodgson, D.J. D'Costa, G.R. Robertson, C. Liddle, Transcriptional regulation of the human CYP3A4 gene by the constitutive androstane receptor, Mol. Pharmacol. 62 (2002) 359–365.

[20] C. Handschin, U.A. Meyer, Induction of drug metabolism: the role of nuclear receptors, Pharmacol. Rev. 55 (2003) 649–673.

[21] J.M. Maglich, C.M. Stoltz, B. Goodwin, D. Hawkins-Brown, J.T. Moore, S.A. Kliewer, Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification, Mol. Pharmacol. 62 (2002) 638–646.

[22] T. Sueyoshi, M. Negishi, Phenobarbital response elements of cytochrome P450 genes and nuclear receptors, Annu. Rev. Pharmacol. Toxicol. 41 (2001) 123–143.

[23] J.M. Maglich, D.J. Parks, L.B. Moore, J.L. Collins, B. Goodwin, A.N. Billin, C.A. Stoltz, S.A. Kliewer, M.H. Lambert, T.M. Willson, J.T. Moore, Identification of a novel human constitutive androstane receptor (CAR) agonist and its use in the identification of CAR target genes, J. Biol. Chem. 278 (2003) 17277–17283.

[24] L. Pichard, E. Raulet, G. Fabre, J.B. Ferrini, J.C. Ourlin, P. Maurel, Human hepatocyte culture, Methods Mol. Biol. 320 (2006) 283–293.

[25] T. Sueyoshi, T. Kawamoto, I. Zelko, P. Honkakoski, M. Negishi, The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene, J. Biol. Chem. 274 (1999) 6043–6046.

[26] L.B. Moore, D.J. Parks, S.A. Jones, R.K. Bledsoe, T.G. Consler, J.B. Stimmel, B. Goodwin, C. Liddle, S.G. Blanchard, T.M. Willson, J.L. Collins, S.A. Kliewer, Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands, J. Biol. Chem. 275 (2000) 15122–15127.

[27] D.J. Mangelsdorf, R.M. Evans, The RXR heterodimers and orphan receptors, Cell 83 (1995) 841–850.

[28] J.P. Aumais, H.S. Lee, C. DeGannes, J. Horsford, J.H. White, Function of directly repeated half-sites as response elements for steroid hormone receptors, J. Biol. Chem. 271 (1996) 12568–12577.

[29] Z. Zhou, J.L. Corden, T.R. Brown, Identification and characterization of a novel androgen response element composed of a direct repeat, J. Biol. Chem. 272 (1997) 8227–8235.

[30] C. Frank, H. Makkonen, T.W. Dunlop, M. Matilainen, S. Vaisanen, C. Carlberg, Identification of pregnane X receptor binding sites in the regulatory regions of genes involved in bile acid homeostasis, J. Mol. Biol. 346 (2005) 505–519.

[31] C.A. Vyhlidal, P.K. Rogan, J.S. Leeder, Development and refinement of pregnane X receptor (PXR) DNA binding site model using information theory: insights into PXR-mediated gene regulation, J. Biol. Chem. 279 (2004) 46779–46786.

[32] C. Danielsson, I.S. Mathiasen, S.Y. James, S. Nayeri, C. Bretting, C.M. Hansen, K.W. Colston, C. Carlberg, Sensitive induction of apoptosis in breast cancer cells by a novel 1,25-dihydroxyvitamin D3 analogue shows relation to promoter selectivity, J. Cell. Biochem. 66 (1997) 552–562.

[33] M. Ellfolk, M. Norlin, K. Wikvall, Isolation and properties of the CYP2D25 promoter: transcriptional regulation by vitamin D3 metabolites, Biochem. Biophys. Res. Commun. 345 (2006) 568–572.

[34] F. Hosseinpour, M. Ellfolk, M. Norlin, K. Wikvall, Phenobarbital suppresses vitamin D(3) 25-hydroxylase expression: A potential new mechanism for drug-induced osteomalacia, Biochem. Biophys. Res. Commun. 357 (2007) 603–607.

[35] Y. Xu, T. Hashizume, M.C. Shuhart, C.L. Davis, W.L. Nelson, T. Sakaki, T.F. Kalhorn, P.B. Watkins, E.G. Schuetz, K.E. Thummel, Intestinal and hepatic CYP3A4 catalyze hydroxylation of 1alpha,25-dihydroxyvitamin D(3): implications for drug-induced osteomalacia, Mol. Pharmacol. 69 (2006) 56–65.

[36] C. Zhou, M. Assem, J.C. Tay, P.B. Watkins, B. Blumberg, E.G. Schuetz, K.E. Thummel, Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia, J. Clin. Invest. 116 (2006) 1703–1712.