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UVB-Induced 1,25(OH)$_2$D$_3$ Production and Vitamin D Activity in Intestinal CaCo-2 Cells and in THP-1 Macrophages Pretreated with a Sterol Δ^7-Reductase Inhibitor

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Abstract Epidermal keratinocytes are able to produce 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$] and induce vitamin D activity upon UVB irradiation. To find out whether this property is keratinocyte specific, we investigated this characteristic in two other cell types, namely intestinal CaCo-2 cells and the macrophage-like differentiated THP-1 cells. THP-1 macrophages and preconfluent CaCo-2 cells contain the vitamin D receptor (VDR), possess 25-hydroxylase (CYP2R1 and CYP27A1) and 1α-hydroxylase (CYP27B1) activity, and survive the low UVB doses essential for vitamin D$_3$ photoproduction. Upon irradiation, 24-hydroxylase (CYP24) mRNA is induced in both cell types pretreated with the sterol Δ^7-reductase inhibitor BM15766 whereby the 7-dehydrocholesterol (7-DHC) content was increased. Transfection studies in CaCo-2 cells with a vitamin D response element-containing construct revealed the involvement of the VDR in this UVB-dependent CYP24 induction. The CYP24 inducing activity in BM15766-pretreated UVB-irradiated CaCo-2 cells and THP-1 macrophages was identified as 1,25(OH)$_2$D$_3$ by combined high-performance liquid chromatography radioimmunoassay. Addition of vitamin D binding protein to the CaCo-2 cells attenuated UVB-induced CYP24 induction suggesting the possibility of a paracrine or autocrine role for the photoproduced 1,25(OH)$_2$D$_3$. In conclusion, preconfluent CaCo-2 cells and THP-1 macrophages are able to induce vitamin D activity upon UVB irradiation and hence combine all parts of the vitamin D photoendocrine system, a characteristic which is therefore not keratinocyte specific. J. Cell. Biochem. 99: 229–240, 2006.

Key words: vitamin D system; ultraviolet; CaCo-2 cells; THP-1 cells

Skin has a unique place in the vitamin D$_3$ system. Skin is not only the site of vitamin D$_3$ photoproduction [Holick, 1988] but also an important target organ for 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$] [Bikle and Pillai, 1993; De Haes et al., 2003; Bikle et al., 2004]. Moreover, it is a site of intensive vitamin D$_3$ metabolism as epidermal keratinocytes express a mitochondrial vitamin D$_3$ 25-hydroxylase (CYP27A1) [Lehmann et al., 1999; Schuessler et al., 2001], the microsomal vitamin D$_3$ 25-hydroxylase (CYP2R1) (unpublished results) and the 25-hydroxyvitamin D$_3$-1α-hydroxylase (CYP27B1) [Bikle et al., 1986a]. These enzymes cooperate as ultraviolet B-(UVB)-induced conversion of exogenous 7-dehydrocholesterol (7-DHC, provitamin D$_3$) to 1,25(OH)$_2$D$_3$ is observed in the transformed cell line HaCaT [Lehmann et al., 2000], in an skin equivalent in vitro [Lehmann et al., 2001] and in human skin in vivo [Lehmann et al., 2003], and the in situ formed 1,25(OH)$_2$D$_3$ might act in a paracrine or autocrine way inducing vitamin D receptor (VDR)-mediated responses [Vantieghem et al., 2005]. Therefore, all parts of the vitamin D system are present

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and act in a concerted way in epidermal keratinocytes.

An important question is whether this characteristic is a hallmark of keratinocytes or shared with other cells. Indeed, vitamin D_3 photoproduction is a photochemical process not subjected to regulation other than 7-DHC availability, and intensity of UVB irradiation [Norman, 1998]. In addition, almost every cell type contains the VDR which is reflected by the immunomodulatory, antiproliferative, and prodifferentiating effects of $1,25(OH)_2D_3$ in addition to the well-known calciotropic actions [Bouillon et al., 1995]. However, vitamin D_3 has almost no VDR activating potential and needs a two-step enzymatic process in order to exert biological effects. The first step is the hepatic formation of 25-hydroxyvitamin D_3 [$25OHD_3$] by the vitamin D_3-25-hydroxylase (CYP27B1 and CYP2R1) [Blunt et al., 1968; Cheng et al., 2003, 2004]; apart from liver, 25-hydroxylase activity is also observed in intestine, adrenal, lung, kidney [Andersson et al., 1989; Usui et al., 1990], bone [Ichikawa et al., 1995], skin [Lehmann et al., 1999], macrophages [Hansson et al., 2003], and in numerous other tissues and cell types [Cheng et al., 2003]. The 1α-hydroxylation of $25OHD_3$ subsequently occurs in kidney by the $25OHD_3$-1α-hydroxylase (CYP27B1) [Lawson et al., 1971], but is also possible in bone [Howard et al., 1981], placenta [Delvin et al., 1985], epidermal keratinocytes [Bikle et al., 1986b], testes [Fu et al., 1997], prostate [Schwartz et al., 1998], macrophages [Monkawa et al., 2000], brain, intestine, adrenal medulla [Zehnder et al., 2001], cervix [Friedrich et al., 2002], and parathyroid glands [Segersten et al., 2002]. Therefore, both vitamin D activation steps are possible at different sites distributed throughout the body.

Accordingly, other cells than epidermal keratinocytes might combine all parts of the photoendocrine vitamin D_3 system and induce vitamin D activity upon exposure to UVB irradiation. We therefore studied THP-1 macrophages and preconfluent CaCo-2 cells, since these cell types are known to be $1,25(OH)_2D_3$ responsive and possess $25OHD_3$-1α-hydroxylase activity [Cross et al., 1997; Bischof et al., 1998; Monkawa et al., 2000; Bareis et al., 2001, 2002; Overbergh et al., 2004], and since macrophages and colon are known to contain vitamin D_3-25-hydroxylase [Theodoropoulos et al., 2001; Hansson et al., 2003].

MATERIALS AND METHODS

Cell Culture

CaCo-2 cells (ATCC, Rockville, MD) were grown in DMEM containing 100 IU/ml penicillin, 100 μg/ml streptomycin, 10% FBS, 2 mM L-glutamine (Invitrogen), and 1% non-essential amino acids (Invitrogen). The cells used in our experiments were between passages 30 and 60. Cells from the human monocytic leukemia cell line THP-1 (ATCC) were grown in RPMI1640 supplemented with 10% fetal calf serum (FBS) (Biochrom AG, Berlin, Germany) and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin) (Invitrogen). Differentiation to the macrophage-like phenotype was induced by incubation with PMA (50 ng/ml) (Sigma, Bornem, Belgium) for 24 h. Treatment with the 1α-hydroxylase stimulating IFNγ was omitted as this resulted in reduced 24-hydroxylase [CYP24] induction upon incubation with $1,25(OH)_2D_3$ [Dusso et al., 1997]. Adherent cells were grown in RPMI1640 supplemented with antibiotics and 0.2% BSA (Invitrogen). Normal human keratinocytes were isolated from foreskins of young donors (less then 6 years) as described [Kitano and Okada, 1983]. Keratinocytes were grown in Keratinocyte Serum Free Medium (Life Technologies, Paisley, Scotland) supplemented with bovine pituitary extract (50 μg/ml) and human epidermal growth factor (5 ng/ml). Third- or fifth-passage cells were used. $1,25(OH)_2D_3$ (gift from Dr. J.P. van de Velde, Solvay, Weesp, The Netherlands), $25(OH)D_3$, $1α(OH)D_3$, vitamin D_3, and ketoconazole (Sigma) were used from a stock in absolute ethanol. Vitamin D binding protein (DBP) was prepared as described [Van Baelen et al., 1980]. BM15766 (kind gift from Boehringer-Mannheim, Mannheim, Germany) was dissolved in DMSO, but final ethanol or DMSO concentrations never exceeded 0.1% and did not affect experiments. Treatment with BM15766 started 24 h before irradiation. Cells were washed twice with PBS, irradiated through a thin film of PBS, and provided fresh medium. Non-irradiated control dishes were handled identically but placed under a dark cloth adjacent to the UVB beam. Three Philips TL20W12 tubes with an emission spectrum between 270 and 400 nm were used as a UVB source and UVB doses were measured with an IL 700 A Research Radiometer (International Light, Newburyport, MA).

RNA Isolation and Northern Blot Analysis

Total RNA was isolated using a commercial kit (RNeasy, Qiagen GmbH, Hilden, Germany). Northern blot analysis was performed as described [Segaert et al., 1997] on 12 μg of total RNA. The following probes were used: human 1α,25-dihydroxyvitamin D_3-24-hydroxylase [CYP24] c-DNA [Chen et al., 1993] and for loading control glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Probes were labeled with α^{32}P-dCTP by the random priming method (RediPrime II, Amersham Biosciences Corp., Piscataway, NJ). Quantification of the bands was established using ImageMaster 1D Elite 3.01 software (Amersham-Pharmacia Biotech).

Real-Time Quantitative PCR (RT-QPCR)

First-strand cDNA was synthesized using SuperScript II reverse transcriptase (Life Technologies, Inc.). PCR reactions were performed in triplicate on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City) using the TaqMan Fast Universal PCR Master Mix (Applied Biosystems) according to the manufacturers manual. Expression levels of *cyp*27A1, *cyp2R1*, and *cyp27B1* were normalized for the hypoxanthin-guanin phosphoribosyltransferase (*HPRT*) gene. Sequences of the forward (FW) and reverse (RV) primers (Eurogentec, Seraing, Belgium) and the 5′-FAM 3′-TAMRA dual labeled probes (TQ) (Eurogentec) were as follows: *Cyp27A1*: GAT CCA TCG GGT TAA TGT TCC A (FW), CAA AGG AAA AGA TGG CAT TCC A (RV), TTC CTC CCC AAG TGG ACT CGC CC (TQ); *Cyp2R1*: CAG CAT TGC TTC AGA GGT TTC A (FW), TTC AGC GTC TTT CAG CAC AGA (RV), ATG ACA TTG CAG CCC CAA CCC TAC C (TQ); *Cyp27B1*: CCC AGA TCC TAA CAC ATT TTG AGG (FW), AAA GGG TGA TGA TGA CAG TCT CTT TC (RV), ACC CAA GAC CCG GAC TGT CCT GGT (TQ); *VDR*: GGA CGC CCA CCA TAA GAC CTA (FW), TGG CTC CCT CCA CCA TCA T (RV), AGT TCC GGC CTC CAG TTC GTG TGA (TQ); *HPRT*: TTA TCA GAC TGA AGA GCT ACT GTA ATG ATC (FW), TTA CCA GTG TCA ATT ATA TCT TCA ACA ATC (RV), TGA GAG AGA TCA TCT CCA CCA ATA ACT TTT ATG TCC C (TQ).

MTT Cytotoxicity Assay

UVB-cytotoxicity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT] uptake (Sigma-Aldrich, Bornem, Belgium). Briefly, cells were seeded in 96-well plates (CaCo-2 cells 4,000 cells/well, THP-1 1×10^4 cells/well, keratinocytes 2,000 cells/well), THP-1 cells were induced to differentiate as described and cells were irradiated with increasing UVB-dose on the second day after seeding. Cells were incubated with MTT (1 mg/ml) in PBS 24 h after UVB irradiation and incubation lasted for 3 h. Cleavage of MTT by dehydrogenase enzymes of metabolically active cells yields a blue formazan product which was dissolved in DMSO. Since many THP-1 macrophages detached during the incubation with MTT, even in de conditions without UVB irradiation, the assay seemed to be toxic and MTT-incubation was limited to 1 h. The absorbance at 570 nm was measured by spectrophotometry.

^{14}C-Acetate Incorporation and Argentation Thin Layer Chromatography [$AgNO_3$ TLC]

Cells were plated in p100 dishes (CaCo-2 0.5×10^6 cells/p100; THP-1 2×10^6 cells/p100) in medium containing 10% FBS. THP-1 cells were induced to differentiate as described. The second day after seeding, CaCo-2 cells were provided FBS-containing or serum free medium, and THP-1 macrophages were provided serum free medium containing 0.2% BSA. Cells were treated with BM15766 10^{-5} M or vehicle and [2-^{14}C]acetate (57 mCi/mmol; 1.5 μCi/dish; Amersham International, Aylesburry, UK) was added to all dishes. After 24 h of incubation, cells were washed twice with PBS, harvested in 1 ml PBS. Samples were extracted twice with *n*-hexane and sterols were separated by means of $AgNO_3$ TLC with chloroform/acetone (9/1) as mobile phase. Silica gel 60 F254 sheets (Merck, Darmstadt, Germany) were soaked in 4% silver nitrate solution in aqueous methanol (9:1) and oven-dried before use. Dried extracts were dissolved in 100 μl acetone and spotted onto the prepared TLC sheets. Sterol precursor pattern is similar as in Shefer et al. [1995].

Transient Transfection and Luciferase Assay

Caco-2 cells were plated into 24-well plates at a density of 7.5×10^4 cells/well and transfected the following day with 0.5 μg of luciferase reporter plasmid containing the rat ANF DR3-type VDRE (kind gift of C. Carlberg, Kuopio, Finland) [Quack et al., 1998] or minimal thymidine kinase reporter plasmid

[Schoenmakers et al., 2000], and 0.025 μg β-galactosidase control plasmid promoter construct using 2 μl/well Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen). Treatment with BM15766 started 6 h after transfection and lasted for 24 h. All cells were irradiated with an UVB-dose of 10 mJ/cm^2 to exclude irradiation effects on gene expression. Cells were harvested in reporter lysis buffer (Promega, Southampton, UK) 24 h after irradiation. Aliquots of 10 μl of cleared lysate were assayed for luciferase activity using a luciferase reporter assay kit from Promega and a Berthold Microlumat LB 96P luminometer. The activity of β-galactosidase (Galacto-light plus, Applied Biosystems, Warrington, UK) was used to normalize for transfection efficiencies.

Analysis of 1,25(OH)$_2$D$_3$

Cells were seeded in p100 dishes (CaCo-2: 0.5×10^6 cells/p100, 10-fold; THP-1: 2×10^6 cells/p100, 10-fold; keratinocytes: 0.5×10^6 cells/p100, twofold), and THP-1 cells were induced to differentiate as described. The following day, treatment with BM15766 started that lasted for 24 h whereupon cells were exposed to UVB irradiation (10 mJ/cm^3). Twenty four hours after irradiation, media were pooled and concentrated in a vacuum centrifuge (Savant). Remaining cells were harvested in 200 μl PBS, pooled and sonicated. Media and homogenates were purified by chromatography on a Sephadex LH-20 column (Pharmacia, Uppsala, Sweden). Subsequent determination of the 1,25(OH)$_2$D$_3$ content was performed by high pressure liquid chromatographic (HPLC) separation followed by radioimmunoassay (RIA) as described previously [Bouillon et al., 1980a]. Obtained volumetric values were converted into fmol/10^6 cells using the average cell number at the time of harvest.

Statistical Analysis

One-way ANOVA was used to compare UVB survival of the different cell types, the luciferase activity of the different conditions in transfection experiments, and to compare 1,25(OH)$_2$D$_3$ content in medium and cellular homogenates. Data are expressed as mean ± SEM. To determine differences from control, *t*-test analyses were applied. *P* values of 0.005 were considered statistically significant.

RESULTS

Preconfluent CaCo-2 Cells and THP-1 Macrophages Are 1,25(OH)$_2$D$_3$ Responsive and Activate Vitamin D$_3$

Preconfluent CaCo-2 cells and THP-1 macrophages contain VDR mRNA (Fig. 1) and, as shown by Northern Blot (Fig. 2), respond to 1,25(OH)$_2$D$_3$ treatment by an induction of CYP24 mRNA of which both onset and intensity were dependent on the dose administered (data not shown). In addition, THP-1 macrophages and preconfluent CaCo-2 cells, like epidermal keratinocytes, contain mRNA of the microsomal (CYP2R1) and the mitochondrial (CYP27-A1) 25-hydroxylase, and the 25OHD$_3$-1α-hydroxylase CYP27B1 (Fig. 1). As shown by RT-QPCR, CYP2R1 is highly expressed in all cell types. To test the functionality of the vitamin D hydroxylases, cells were treated with 1αOHD$_3$ and 25OHD$_3$, and CYP24 expression was used as a marker for VDR transactivation and therefore for vitamin D$_3$ metabolite activation (Fig. 2). A clear induction of CYP24 mRNA was seen 24 h after incubation with 1αOHD$_3$ and 25OHD$_3$, which was attenuated when the universal cytochrome P450 enzyme inhibitor ketoconazole was added 6 h before stimulation with the vitamin D$_3$ metabolites (Fig. 2). In addition, stimulation with vitamin D$_3$, that has almost no intrinsic VDR activating potential (RCI $_{\text{chicken VDR}}$, vitamin D$_3$ = 0.0001) [Bouillon et al., 1995], resulted in a significant CYP24 mRNA induction, which was blocked by ketoconazole.

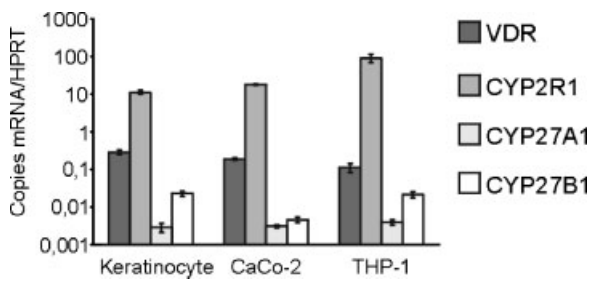

Fig. 1. Preconfluent CaCo-2 cells and THP-1 macrophages contain all components of the vitamin D system. Epidermal keratinocytes, preconfluent CaCo-2 cells, and THP-1 cells were cultured as described. Two micrograms RNA was used for c-DNA synthesis and analyzed for the expression of the indicated gene by RT-QPCR. Data represented are mean values (±SEM) of at least three independent samples.

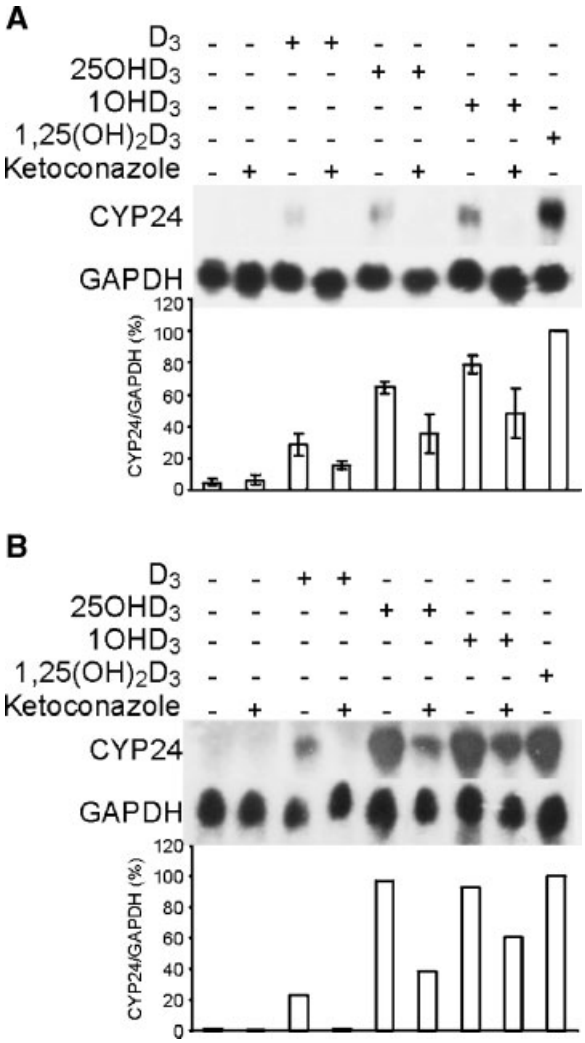

Fig. 2. CYP24 induction in preconfluent CaCo-2 cells (**A**) and THP-1 macrophages (**B**) upon stimulation with vitamin D_3, 25OHD_3, 1αOHD_3, and 1,25(OH)$_2$D$_3$. Cells were stimulated with the indicated dose vitamin D_3 metabolite (10^{-7} M) for 24 h in absence or in presence of ketoconazole 10^{-5} M added 6 h before stimulation with the vitamin D_3 metabolites. Cells were harvested and total RNA was isolated. Twelve micrograms of RNA was used for Northern Blot analysis and hybridized with the indicated radiolabeled probes. A set of representative data of at least two independent experiments is presented.

Preconfluent CaCo-2 and THP-1 Macrophages Survive Low-Dose UVB Irradiation

For UVB-induced vitamin D activity, it is necessary that the cells survive at least the minimal UVB dose essential for vitamin D_3 photoproduction. MTT-cytotoxicity assay revealed that survival of THP-1 macrophages and preconfluent CaCo-2 cells decreased after exposure to an increasing UVB dose, like in epidermal keratinocytes (Fig. 3). However, the

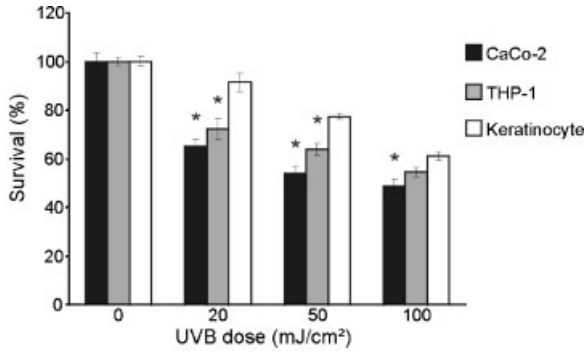

Fig. 3. MTT survival: Preconfluent CaCo-2 cells and THP-1 macrophages survive UVB irradiation to an equal extent as epidermal keratinocytes. MTT survival assay of preconfluent CaCo-2 cells, THP-1 macrophages, and epidermal keratinocytes 24 h after exposure to the indicated UVB dose. Data represented are mean values (±SEM) of at least three independent experiments. *$P < 0.005$ versus keratinocyte.

decrease is more pronounced for preconfluent CaCo-2 cells and THP-1 macrophages than for epidermal keratinocytes. Upon exposure to 20 mJ/cm^3, the number of viable CaCo-2 or THP-1 cells as measured by MTT is reduced to 65% and 72%, respectively, compared to control cells. Compared to keratinocyte survival, these rates correspond to exposure to UVB doses of 100 or 50 mJ/cm^2, respectively. In order to maintain a sufficient fraction of viable cells while reducing apoptotic effects, UVB exposure of preconfluent CaCo-2 cells and THP-1 macrophages was limited to 10 mJ/cm^2 in further experiments. Indeed, increasing the UVB-dose in epidermal keratinocytes initially increased but subsequently reduced CYP24 mRNA induction when UVB dose further increased (unpublished results).

CaCo-2 Cells and THP-1 Macrophages Pretreated With an Inhibitor of the Sterol Δ^7-Reductase Induce CYP24 mRNA Upon UVB Irradiation

CYP24 is considered as one of the most sensitive 1,25(OH)$_2$D$_3$ responsive genes and its transcription is often used as a marker for the presence of active vitamin D metabolites [Makin et al., 1989]. Therefore, production of active vitamin D metabolites in preconfluent CaCo-2 cells or in THP-1 macrophages upon UVB irradiation might be reflected in the induction of CYP24 mRNA. However, we could not detect any CYP24 mRNA by Northern Blot 24 and 48 h after exposure to 10 mJ/cm^2 UVB irradiation (Fig. 4A,C). As UVB-induced vitamin D_3 photoproduction is a completely

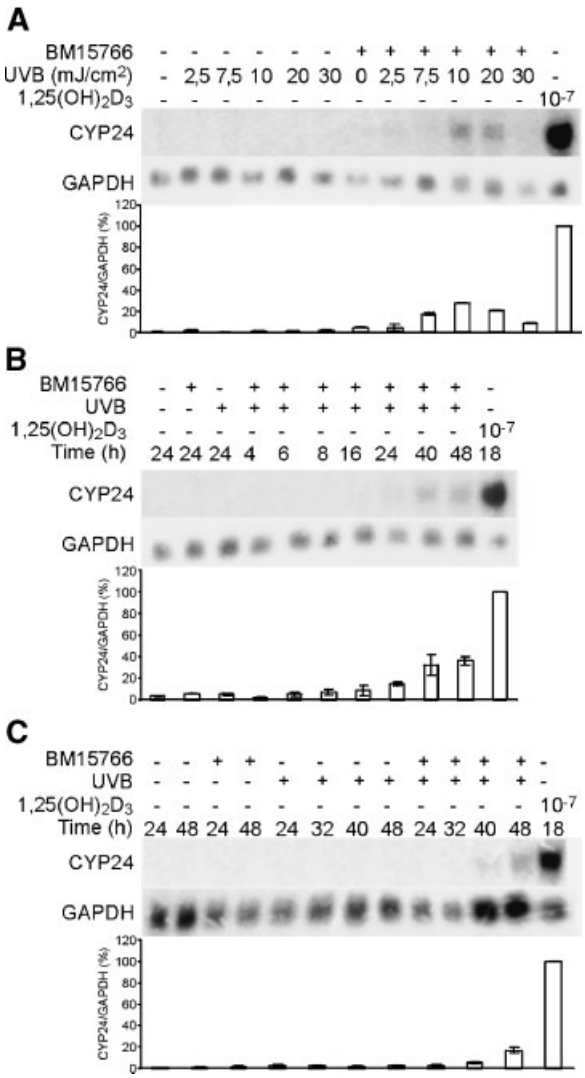

Fig. 4. UVB induces CYP24 mRNA expression in preconfluent CaCo-2 cells (**A**, **B**) and THP-1 macrophages (**C**) pretreated with BM15766. Cells were treated for 24 h with BM15766 10^{-5} M before exposure to a UVB dose of 10 mJ/cm^2 (A, C) or with the indicated UVB dose (B). Cells were harvested at the indicated time after irradiation (A, C), 40 h after irradiation (C) or 18 h after stimulation with 1,25(OH)$_2$D$_3$, and total RNA was isolated. Twelve micrograms of RNA was used for Northern Blot analysis and hybridized with the indicated radiolabeled probes. A set of representative data of at least two independent experiments is presented.

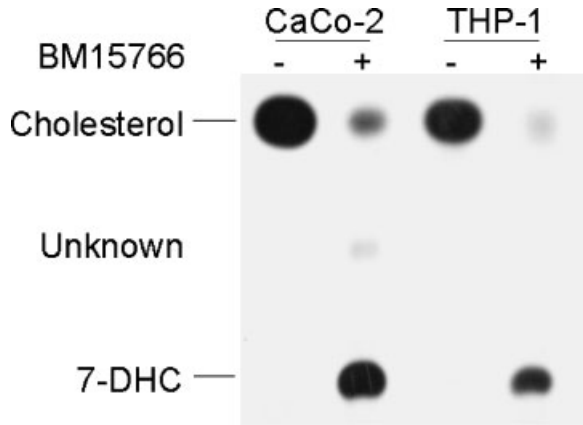

Fig. 5. BM15766-treatment of preconfluent CaCo-2 cells and THP-1 macrophages results in accumulation of 7-DHC. Preconfluent CaCo-2 cells cultured in serum-containing or serum free medium were treated with BM15766 10^{-5} M in the presence of ^{14}C-acetate for 24 h. THP-1 cells were induced to differentiate as described and treated with BM15766 10^{-5} M in the presence of ^{14}C-acetate for 24 h. Cells were harvested in PBS and extracted with ethylacetate/cyclohexane (1:1 for TLC analysis). One representative autoradiogram is presented.

non-enzymatic photochemical process not subjected to regulation other than substrate availability and intensity of UVB irradiation [Norman, 1998], we assumed that the low 7-DHC content in preconfluent CaCo-2 cells or THP-1 macrophages could be a limiting factor [Nemanic et al., 1985]. In order to increase the endogenous 7-DHC stores and therefore the amount of vitamin D$_3$ produced upon UVB irradiation, BM15766, a sterol Δ^7-reductase inhibitor [Shefer et al., 1998], was added to the cells. This resulted in a clear 7-DHC accumulation 24 h after incubation with BM15766 (Fig. 5) as measured by $AgNO_3$ TLC analysis of lipid extracts after ^{14}C-acetate incorporation. In addition, a marked induction of CYP24 mRNA was seen in BM15766-treated CaCo-2 cells that started at 16 h after irradiation and reached a peak intensity at 40 h after irradiation (Fig. 4A). This UVB-induced CYP24 was UVB dose dependent but reached a peak intensity between 10 and 20 mJ/cm^2 (Fig. 4B). Growing the CaCo-2 cells in Serum Free Keratinocyte medium after irradiation enhanced the CYP24 mRNA induction probably due to the presence of stimulatory factors in the keratinocyte medium. The UVB-induced CYP24 was also seen in THP-1 macrophages (Fig. 4C), but was not seen before 40 h after irradiation. Culture of THP-1 macrophages in Serum Free Keratinocyte medium reduced the 1,25(OH)$_2$D$_3$-induced CYP24 induction (data not shown). However, onset of UVB-induced CYP24 was delayed and intensity was attenuated in both cell types as compared to UVB-induced CYP24 in epidermal keratinocytes [Vantieghem et al., 2005].

Addition of ketoconazole prior to UVB irradiation abrogated the CYP24 expression in preconfluent CaCo-2 cells, but addition 16 h after irradiation enhanced the induction of

CYP24 mRNA (data not shown). This time-dependent effect of ketoconazole suggests that the CYP24-inducing activity (presumably active vitamin D metabolites) depends on CYP450 enzymes both for its synthesis as well as for its degradation.

Induction of VDR-Mediated Transcription in CaCo-2 Cells Pretreated With an Inhibitor of the Sterol Δ^7-Reductase by UVB Irradiation

In order to investigate the involvement of the VDR in the UVB-induced CYP24 transcription, preconfluent CaCo-2 cells were transiently transfected with a VDRE-luciferase reporter construct. This resulted in a dose-dependent induction of luciferase activity upon 1,25(OH)$_2$D$_3$ stimulation and insignificantly increased luciferase activity upon UVB irradiation of BM15766-pretreated CaCo-2 cells as compared to UVB-irradiated BM15766-deprived CaCo-2 cells (Fig. 6).

Identification of 1,25(OH)$_2$D$_3$ in UVB-Irradiated CaCo-2 Cells and THP-1 Cells Pretreated With an Inhibitor of the Sterol Δ^7-Reductase

Marked amounts of 1,25(OH)$_2$D$_3$ were detected by HPLC/RIA in medium and cellular homogenates of BM15766-pretreated preconfluent CaCo-2 cells 24 h after irradiation with 10 mJ/cm^2 UVB (Table I). No 1,25(OH)$_2$D$_3$ was found in control medium and very minute quantities that only hardly exceed the detection limit cells were found in cellular homogenates.

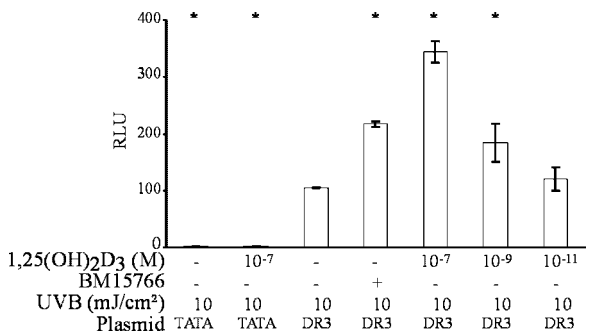

Fig. 6. UVB induces VDR-mediated transcription in preconfluent CaCo-2 cells pretreated with BM15766. Cells were transfected with either VDRE-driven luciferase reporter construct (VDRE) or minimal reporter construct (TATA). Treatment with BM15766 10^{-5} M lasted for 24 h whereupon cells were irradiated with UVB (10 mJ/cm^2). Forty hours after irradiation or 24 h after 1,25(OH)$_2$D$_3$ stimulation, cells were harvested with reporter lysis buffer and the lysates were analyzed for luciferase activity (RLU, relative luciferase activity). Data represented are mean values (±SEM) of at least three independent experiments performed in duplicate. *$P < 0.005$ versus control.

TABLE I. Effect of BM15766-Treatment and UVB Irradiation on the 1,25(OH)$_2$D$_3$ Content (fmol/10^6 cells) in Medium and Cellular Homogenates of Preconfluent CaCo-2 cells[a,b], THP-1 Macrophages,[a,c] and Primary Epidermal Keratinocytes[a,b]

CaCo-2 cell[d]	Medium	Homogenate
Control	—	1.7 (0.5)
BM15766[e]	—	1.9 (0.6)
UVB 10 mJ/cm^2	—	1.9 (0.5)
BM15766 + UVB 10 mJ/cm^2	4.8* (0.7)	7.8* (0.7)
THP-1 macrophages[f]	Medium	Homogenate
Control	—	3.5
BM15766[e]	—	2.8
UVB 10 mJ/cm^2	—	2.5
BM15766 + UVB 10mJ/cm^2	—	6.25
PHK[g]	Medium	Homogenate
Control	—	—
BM15766 + UVB 10 mJ/cm^2	—	13.7* (3.1)

Differences in detection limit result from differences in sample volume and cell numbers.
[a]1,25(OH)$_2$D$_3$ content was measured by HPLC/RIA 24 h after irradiation or 48 h after BM15766 treatment.
[b]Results represent mean (SEM) of two experiments.
[c]Results of one representative experiment.
[d]Detection limit 1 fmol/10^6 cells.
[e]BM15766 treatment (10^{-5} M) lasted 24 h.
[f]Detection limit 2 fmol/10^6 cells.
[g]Detection limit 9 fmol/10^6 cells.
*$P < 0.001$ versus control.

In addition, elevated 1,25(OH)$_2$D$_3$ was detected in cellular homogenates of BM15766-pretreated THP-1 macrophages, but not in conditioned medium. However, 1,25(OH)$_2$D$_3$ levels were lower than obtained in epidermal keratinocytes.

Vitamin D Binding Protein (DBP) Abrogates CYP24 mRNA Induction in UVB Irradiated CaCo-2 Cells Pretreated With a Sterol Δ^7-Reductase Inhibitor

We present evidence that UVB irradiation induces production of 1,25(OH)$_2$D$_3$ and expression of vitamin D-dependent genes. Since these processes occur within the cell, these results may reflect an intracrine pathway. However, addition of serum or vitamin D binding protein (DBP), a serum protein with very high and specific binding affinity for vitamin D metabolites (K_a, 1,25(OH)$_2$D$_3$ 1.5×10^{-7} M, K_a, 25OHD$_3$ 5×10^{-8} M) [Bouillon et al., 1980b], in physiological dose (300 μg/ml) to the culture medium after UVB irradiation significantly attenuated CYP24 mRNA induction in preconfluent CaCo-2 cells (Fig. 7). DBP does not affect VDR transactivation as preconfluent CaCo-2 cells lack receptors for DBP and as passive diffusion of DBP through the plasma membrane is quantitatively insignificant [Ramanujam et al., 1991; Xu and Fyfe, 2000; Nykjaer et al., 2001]. Therefore, the blocking effect of DBP on

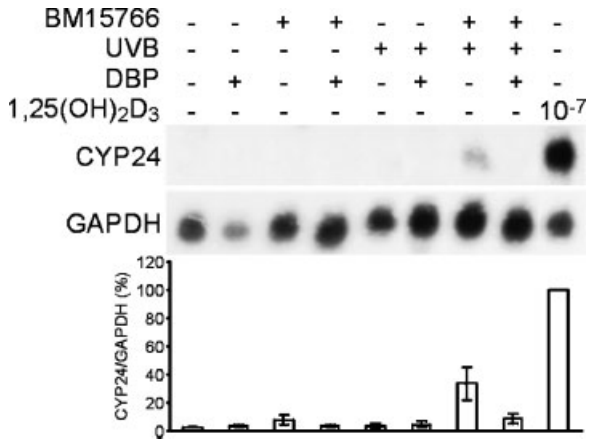

Fig. 7. Evidence for a paracrine or autocrine role for photoproduced vitamin D metabolites. Cells were treated for 24 h with BM15766 10^{-5} M before exposure to a UVB-dose of 10 mJ/cm^2. Immediately after irradiation, 300 μg/ml DBP was added. Cells were harvested 40 h after irradiation or 24 h after 1,25(OH)$_2$D$_3$ stimulation and total RNA was isolated. Twelve micrograms RNA was used for Northern Blot analysis and hybridized with the indicated radiolabeled probes. A set of representative data of at least two independent experiments is presented.

UVB-induced CYP24 refers to secretion of 1,25(OH)$_2$D$_3$ into the medium before exerting vitamin D effects on the BM15766-pretreated UVB-irradiated preconfluent CaCo-2 cells although secretion of 25OHD$_3$ before final activation into 1,25(OH)$_2$ D$_3$ cannot be excluded. However, 1,25(OH)$_2$D$_3$ was also found in the medium of BM15766-pretreated UVB-irradiated CaCo-2 cells (Table I). Hence, these results feed the contention that photoproduced vitamin D$_3$ metabolites mainly acts as an autocrine or paracrine rather than an intracrine factor.

DISCUSSION

In this study, we show that the coordinated presence of all parts of the vitamin D system in epidermal keratinocytes is not keratinocyte specific. At least, the intestinal CaCo-2 cells and THP-1 macrophages share this feature as these cells are also able to induce genomic vitamin D activity upon UVB irradiation, and other cells might do so as well.

An essential prerequisite for UVB-induced vitamin D activity is the presence and functionality of all parts of the photoendocrine vitamin D system. Upon exposure to physiological UVB-doses, vitamin D$_3$ needs to be formed from 7-DHC and further activated into 1,25(OH)$_2$D$_3$. In addition, the cells need to respond to the locally produced 1,25(OH)$_2$D$_3$. As almost every cell type contains a VDR and is 1,25(OH)$_2$D$_3$ responsive, this requisite is probably not critical. Likewise, vitamin D$_3$ photoproduction is a non-enzymatic photochemical process only depending on substrate availability and intensity of UVB irradiation [Norman, 1998]. For this reason, we assumed vitamin D$_3$ photoproduction to be possible in each cell given the presence of enough 7-DHC. To achieve this, cells were treated with an inhibitor of the sterol Δ^7-reductase, the last enzyme in cholesterol biosynthesis converting 7-DHC into cholesterol [Shefer et al., 1998]. On the other hand, the activation of vitamin D$_3$ into 1,25(OH)$_2$D$_3$ might be more crucial in UVB-dependent 1,25(OH)$_2$D$_3$ synthesis. Indeed, although extrahepatic 25-hydroxylation of vitamin D is widely distributed throughout many tissues and cell types, extrarenal 1α-hydroxylase activity is less distributed and seems to be limited to epithelial cells, monocytes and antigen presenting cells [Hewison et al., 2004]. Therefore, in search for another cell type inducing vitamin D activity upon UVB irradiation, we focused on THP-1 macrophages and CaCo-2 cells, both cell types with known 1α-hydroxylase activity [Cross et al., 1997; Monkawa et al., 2000; Overbergh et al., 2004]. These cells were shown to possess mRNA of the microsomal (CYP2R1) and the mitochondrial (CYP27A1) vitamin D$_3$-25-hydroxylase, and induce CYP24 upon stimulation with vitamin D$_3$, indicating that these cells can indeed activate vitamin D$_3$. Moreover, mRNA of CYP2R1 was far more abundant than mRNA of CYP27A1 so that 25-hydroxylase activity might be executed mainly by CYP2R1, which is considered as the physiological vitamin D$_3$-25-hydroxylase [Cheng et al., 2004].

Nevertheless, for UVB-induced vitamin D activity, all parts of the vitamin D$_3$ system need to cooperate after UVB exposure and therefore a minimal UVB survival is essential. The sun is human's physiological source of UVB irradiation and only skin and eyes are exposed to it in vivo, both having a huge protective capacity to deal with this stressor [Otto et al., 1999; Adachi et al., 2003; Cejkova et al., 2004]. Besides, each cell possesses to a variable extent a complex network of repair systems keeping the DNA under continuous surveillance or dealing with the oxidative damage, and hence might preserve its capacity to activate vitamin D$_3$ and induce vitamin D activity upon exposure to the low UVB dose necessary for vitamin D$_3$

photoproduction [Aw, 1999; Sanders et al., 2004]. However, intestinal CaCo-2 cell and THP-1 macrophage UVB-survival decrease upon exposure to increasing UVB doses in a more pronounced way than keratinocyte UVB-survival reflecting reduced capacity to deal with UVB irradiation. Anyhow, since UVB-induced vitamin D activity in epidermal keratinocytes was detectable already at low UVB dose [Vantieghem et al., 2005], we considered the study of the photoendocrine vitamin D_3 system in preconfluent CaCo-2 cells and THP-1 macrophages to be challenging.

Indeed, we succeeded in detecting CYP24 mRNA in both cell types upon exposure to low-dose UVB irradiation in both cell types. However, increasing endogenous 7-DHC content by pretreatment with the sterol Δ^7-reductase inhibitor BM15766 was required. In addition, UVB-induced CYP24 in preconfluent CaCo-2 cells or THP-1 macrophages was observed at later time points, which might reflect the fact that CYP24 mRNA is induced at later time points after 1,25$(OH)_2D_3$ administration in both cell types (data not shown) [Tomon et al., 1990; Dusso et al., 1997] or reduced vitamin D_3 activation capacity upon UVB irradiation.

In addition, UVB-induced CYP24 was clearly less intense as compared to UVB-induced CYP24 in epidermal keratinocytes, but this might be a consequence of reduced 1,25$(OH)_2D_3$ sensitivity, reduced 7-DHC accumulation upon BM15766 pretreatment, or reduced vitamin D_3 activation potential upon UVB irradiation. Transfection experiments in preconfluent CaCo-2 cells in which a VDRE-containing luciferase reporter construct was introduced revealed the involvement of the VDR and therefore, UVB-induced CYP24 reflects UVB-induced vitamin D activity. Indeed, CYP24 is one of the most sensitive 1,25$(OH)_2D_3$ responsive genes [Makin et al., 1989; Chen et al., 1994], and its induction represents a negative feedback mechanism in 1,25$(OH)_2D_3$-dependent gene expression reducing the availability of VDR ligand [Chen et al., 1994]. Furthermore, the modulating effect of ketoconazole supports the contention that VDR transactivation took place by the generation of photoproducts (like vitamin D_3) that need activation in order to act as a VDR ligand. Nevertheless, the identification of 1,25$(OH)_2D_3$ in the medium and cellular homogenates of BM15766-pretreated UVB-irradiated CaCo-2 cells, and THP-1 macrophages ultimately confirmed that the UVB-induced CYP24 in preconfluent CaCo-2 cells and in BM15766-pretreated THP-1 macrophages was the result of vitamin D_3 photoproduction, subsequent conversion into 1,25$(OH)_2D_3$ and VDR transactivation. In analogy to UVB-induced vitamin D activity, UVB-induced 1,25$(OH)_2D_3$ production in preconfluent CaCo-2 cells or THP-1 macrophages was less abundant than epidermal keratinocytes.

In addition, we show the possibility of a paracrine or autocrine role rather than a solely intracrine role for the photoproduced vitamin D_3 metabolites in preconfluent CaCo-2 cells. Addition of DBP blocked UVB-induced vitamin D activity and 1,25$(OH)_2D_3$ was also detected extracellularly in medium of BM15766-pretreated UVB-irradiated CaCo-2 cells.

As only the skin is normally exposed to UVB, keratinocytes and dermal fibroblasts are the source of cutaneous vitamin D production. Monocytes, macrophages, and dendritic cells present in normal and especially in inflammatory skin lesions, however might be direct UVB targets and their complete vitamin D system could be involved in the beneficial effects of phototherapy of inflammatory skin lesions. Intestinal cells, on the other hand, are not exposed to UVB-irradiation in vivo. Therefore, our data with CaCo-2 cells lack physiological relevance. However, our main goal was to determine whether UVB-induced vitamin D activity was a unique feature of epidermal keratinocytes, for which they are highly specialized, or whether this only took place because epidermal keratinocytes happen to be exposed to UVB. Since intestinal CaCo-2 cells contain vitamin D_3 25-hydroxylase and 25-hydroxyvitamin D_3 1α-hydroxylase activity, these cells proved useful to reach that goal.

In conclusion, we show that the unique cooperation of all parts of the photoendocrine vitamin D system and hence the ability to induce vitamin D activity upon UVB exposure is not an exclusive hallmark of epidermal keratinocytes. At least intestinal CaCo-2 cells and THP-1 macrophages share this characteristic, and other cells might do so as well since the two requirements, the intracellular combination of all components of the vitamin D_3 system and the sufficient UVB survival, are not keratinocyte specific.

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REFERENCES

Adachi M, Gazel A, Pintucci G, Shuck A, Shifteh S, Ginsburg D, Rao LS, Kaneko T, Freedberg IM, Tamaki K, Blumenberg M. 2003. Specificity in stress response: Epidermal keratinocytes exhibit specialized UV-responsive signal transduction pathways. DNA Cell Biol 22:665–677.

Andersson S, Davis DL, Dahlback H, Jornvall H, Russell DW. 1989. Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. J Biol Chem 264:8222–8229.

Aw TY. 1999. Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. Am J Clin Nutr 70:557–565.

Bareis P, Bises G, Bischof MG, Cross HS, Peterlik M. 2001. 25-hydroxy-vitamin d metabolism in human colon cancer cells during tumor progression. Biochem Biophys Res Commun 285:1012–1017.

Bareis P, Kallay E, Bischof MG, Bises G, Hofer H, Potzi C, Manhardt T, Bland R, Cross HS. 2002. Clonal differences in expression of 25-hydroxyvitamin D(3)-1alpha-hydroxylase, of 25-hydroxyvitamin D(3)-24-hydroxylase, and of the vitamin D receptor in human colon carcinoma cells: Effects of epidermal growth factor and 1alpha,25-dihydroxyvitamin D(3). Exp Cell Res 276:320–327.

Bikle DD, Pillai S. 1993. Vitamin D, calcium, and epidermal differentiation. Endocr Rev 14:3–19.

Bikle DD, Nemanic MK, Gee E, Elias P. 1986a. 1,25-Dihydroxyvitamin D3 production by human keratinocytes. Kinetics and regulation. J Clin Invest 78:557–566.

Bikle DD, Nemanic MK, Whitney JO, Elias PW. 1986b. Neonatal human foreskin keratinocytes produce 1,25-dihydroxyvitamin D3. Biochemistry 25:1545–1548.

Bikle DD, Chang S, Crumrine D, Elalieh H, Man MQ, Choi EH, Dardenne O, Xie Z, Arnaud RS, Feingold K, Elias PM. 2004. 25 Hydroxyvitamin D 1 alpha-hydroxylase is required for optimal epidermal differentiation and permeability barrier homeostasis. J Invest Dermatol 122:984–992.

Bischof MG, Siu-Caldera ML, Weiskopf A, Vouros P, Cross HS, Peterlik M, Reddy GS. 1998. Differentiation-related pathways of 1 alpha,25-dihydroxycholecalciferol metabolism in human colon adenocarcinoma-derived Caco-2 cells: Production of 1 alpha,25-dihydroxy-3epi-cholecalciferol. Exp Cell Res 241:194–201.

Blunt JW, DeLuca HF, Schnoes HK. 1968. 25-hydroxycholecalciferol: A biologically active metabolite of vitamin D3. Biochemistry 7:3317–3322.

Bouillon R, De Moor P, Baggiolini EG, Uskokovic MR. 1980a. A radioimmunoassay for 1,25-dihydroxycholecalciferol. Clin Chem 26:562–567.

Bouillon R, Van Baelen H, De Moor P. 1980b. Comparative study of the affinity of the serum vitamin D-binding protein. J Steroid Biochem 13:1029–1034.

Bouillon R, Okamura WH, Norman AW. 1995. Structure-function relationships in the vitamin D endocrine system. Endocr Rev 16:200–257.

Cejkova J, Stipek S, Crkovska J, Ardan T, Platenik J, Cejka C, Midelfart A. 2004. UV Rays, the prooxidant/antioxidant imbalance in the cornea and oxidative eye damage. Physiol Res 53:1–10.

Chen KS, Prahl JM, DeLuca HF. 1993. Isolation and expression of human 1,25-dihydroxyvitamin D3 24-hydroxylase cDNA. Proc Natl Acad Sci USA 90:4543–4547.

Chen ML, Heinrich G, Ohyama YI, Okuda K, Omdahl JL, Chen TC, Holick MF. 1994. Expression of 25-hydroxyvitamin D3-24-hydroxylase mRNA in cultured human keratinocytes. Proc Soc Exp Biol Med 207:57–61.

Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. 2003. De-orphanization of cytochrome P450 2R1: A microsomal vitamin D 25-hydroxilase. J Biol Chem 278:38084–38093.

Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. 2004. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 101:7711–7715.

Cross HS, Peterlik M, Reddy GS, Schuster I. 1997. Vitamin D metabolism in human colon adenocarcinoma-derived Caco-2 cells: Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase activity and regulation of side-chain metabolism. J Steroid Biochem Mol Biol 62:21–28.

De Haes P, Garmyn M, Degreef H, Vantieghem K, Bouillon R, Segaert S. 2003. 1,25-Dihydroxyvitamin D3 inhibits ultraviolet B-induced apoptosis, Jun kinase activation, and interleukin-6 production in primary human keratinocytes. J Cell Biochem 89:663–673.

Delvin EE, Arabian A, Glorieux FH, Mamer OA. 1985. In vitro metabolism of 25-hydroxycholecalciferol by isolated cells from human decidua. J Clin Endocrinol Metab 60:880–885.

Dusso AS, Kamimura S, Gallieni M, Zhong M, Negrea L, Shapiro S, Slatopolsky E. 1997. Gamma-Interferon-induced resistance to 1,25-(OH)2 D3 in human monocytes and macrophages: A mechanism for the hypercalcemia of various granulomatoses. J Clin Endocrinol Metab 82:2222–2232.

Friedrich M, Villena-Heinsen C, Axt-Fliedner R, Meyberg R, Tilgen W, Schmidt W, Reichrath J. 2002. Analysis of 25-hydroxyvitamin D3-1alpha-hydroxylase in cervical tissue. Anticancer Res 22:183–186.

Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, Portale AA. 1997. Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. Mol Endocrinol 11:1961–1970.

Hansson M, Ellis E, Hunt MC, Schmitz G, Babiker A. 2003. Marked induction of sterol 27-hydroxylase activity and mRNA levels during differentiation of human cultured monocytes into macrophages. Biochim Biophys Acta 1593:283–289.

Hewison M, Zehnder D, Chakraverty R, Adams JS. 2004. Vitamin D and barrier function: A novel role for extra-renal 1 alpha-hydroxylase. Mol Cell Endocrinol 215:31–38.

Holick MF. 1988. Skin: Site of the synthesis of vitamin D and a target tissue for the active form, 1,25-dihydroxyvitamin D3. Ann NY Acad Sci 548:14–26.

Howard GA, Turner RT, Sherrard DJ, Baylink DJ. 1981. Human bone cells in culture metabolize 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3. J Biol Chem 256:7738–7740.

Ichikawa F, Sato K, Nanjo M, Nishii Y, Shinki T, Takahashi N, Suda T. 1995. Mouse primary osteoblasts express vitamin D3 25-hydroxylase mRNA and convert 1 alpha-hydroxyvitamin D3 into 1 alpha,25-dihydroxyvitamin D3. Bone 16:129–135.

Kitano Y, Okada N. 1983. Separation of the epidermal sheet by dispase. Br J Dermatol 108:555–560.

Lawson DE, Fraser DR, Kodicek E, Morris HR, Williams DH. 1971. Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. Nature 230:228–230.

Lehmann B, Tiebel O, Meurer M. 1999. Expression of vitamin D3 25-hydroxylase (CYP27) mRNA after induction by vitamin D3 or UVB radiation in keratinocytes of human skin equivalents—a preliminary study. Arch Dermatol Res 291:507–510.

Lehmann B, Knuschke P, Meurer M. 2000. UVB-induced conversion of 7-dehydrocholesterol to 1 alpha,25-dihydroxyvitamin D3 (calcitriol) in the human keratinocyte line HaCaT. Photochem Photobiol 72:803–809.

Lehmann B, Genehr T, Knuschke P, Pietzsch J, Meurer M. 2001. UVB-induced conversion of 7-dehydrocholesterol to 1alpha,25-dihydroxyvitamin D3 in an in vitro human skin equivalent model. J Invest Dermatol 117:1179–1185.

Lehmann B, Sauter W, Knuschke P, Dressler S, Meurer M. 2003. Demonstration of UVB-induced synthesis of 1 alpha,25-dihydroxyvitamin D3 (calcitriol) in human skin by microdialysis. Arch Dermatol Res 295:24–28.

Makin G, Lohnes D, Byford V, Ray R, Jones G. 1989. Target cell metabolism of 1,25-dihydroxyvitamin D3 to calcitroic acid. Evidence for a pathway in kidney and bone involving 24-oxidation. Biochem J 262:173–180.

Monkawa T, Yoshida T, Hayashi M, Saruta T. 2000. Identification of 25-hydroxyvitamin D3 1alpha-hydroxylase gene expression in macrophages. Kidney Int 58:559–568.

Nemanic MK, Whitney J, Elias PM. 1985. In vitro synthesis of vitamin D-3 by cultured human keratinocytes and fibroblasts: Action spectrum and effect of AY-9944. Biochim Biophys Acta 841:267–277.

Norman AW. 1998. Sunlight, season, skin pigmentation, vitamin D, and 25-hydroxyvitamin D: Integral components of the vitamin D endocrine system. Am J Clin Nutr 67:1108–1110.

Nykjaer A, Fyfe JC, Kozyraki R, Leheste JR, Jacobsen C, Nielsen MS, Verroust PJ, Aminoff M, de la CA, Moestrup SK, Ray R, Gliemann J, Willnow TE, Christensen EI. 2001. Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D(3). Proc Natl Acad Sci USA 98:13895–13900.

Otto AI, Riou L, Marionnet C, Mori T, Sarasin A, Magnaldo T. 1999. Differential behaviors toward ultraviolet A and B radiation of fibroblasts and keratinocytes from normal and DNA-repair-deficient patients. Cancer Res 59:1212–1218.

Overbergh L, Stoffels K, Valckx D, Giulietti A, Bouillon R, Mathieu C. 2004. Regulation of 25-hydroxyvitamin d-1alpha-hydroxylase by IFNgamma in human monocytic THP1 cells. J Steroid Biochem Mol Biol 89-90:453–455.

Quack M, Szafranski K, Rouvinen J, Carlberg C. 1998. The role of the T-box for the function of the vitamin D receptor on different types of response elements. Nucleic Acids Res 26:5372–5378.

Ramanujam KS, Seetharam S, Ramasamy M, Seetharam B. 1991. Expression of cobalamin transport proteins and cobalamin transcytosis by colon adenocarcinoma cells. Am J Physiol 260:G416–G422.

Sanders LM, Henderson CE, Hong MY, Barhoumi R, Burghardt RC, Carroll RJ, Turner ND, Chapkin RS, Lupton JR. 2004. Pro-oxidant environment of the colon compared to the small intestine may contribute to greater cancer susceptibility. Cancer Lett 208:155–161.

Schoenmakers E, Verrijdt G, Peeters B, Verhoeven G, Rombauts W, Claessens F. 2000. Differences in DNA binding characteristics of the androgen and glucocorticoid receptors can determine hormone-specific responses. J Biol Chem 275:12290–12297.

Schuessler M, Astecker N, Herzig G, Vorisek G, Schuster I. 2001. Skin is an autonomous organ in synthesis, two-step activation and degradation of vitamin D(3): CYP27 in epidermis completes the set of essential vitamin D(3)-hydroxylases. Steroids 66:399–408.

Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL, Holick MF. 1998. Human prostate cells synthesize 1,25-dihydroxyvitamin D3 from 25-hydroxyvitamin D3. Cancer Epidemiol Biomarkers Prev 7:391–395.

Segaert S, Garmyn M, Degreef H, Bouillon R. 1997. Retinoic acid modulates the anti-proliferative effect of 1,25-dihydroxyvitamin D3 in cultured human epidermal keratinocytes. J Invest Dermatol 109:46–54.

Segersten U, Correa P, Hewison M, Hellman P, Dralle H, Carling T, Akerstrom G, Westin G. 2002. 25-hydroxyvitamin D(3)-1alpha-hydroxylase expression in normal and pathological parathyroid glands. J Clin Endocrinol Metab 87:2967–2972.

Shefer S, Salen G, Batta AK, Honda A, Tint GS, Irons M, Elias ER, Chen TC, Holick MF. 1995. Markedly inhibited 7-dehydrocholesterol-delta 7-reductase activity in liver microsomes from Smith-Lemli-Opitz homozygotes. J Clin Invest 96:1779–1785.

Shefer S, Salen G, Honda A, Batta AK, Nguyen LB, Tint GS, Ioannou YA, Desnick R. 1998. Regulation of rat hepatic 3beta-hydroxysterol delta7-reductase: Substrate specificity, competitive and non-competitive inhibition, and phosphorylation/dephosphorylation. J Lipid Res 39:2471–2476.

Theodoropoulos C, Demers C, Mirshahi A, Gascon-Barre M. 2001. 1,25-Dihydroxyvitamin D(3) downregulates the rat intestinal vitamin D(3)-25-hydroxylase CYP27A. Am J Physiol Endocrinol Metab 281:E315–E325.

Tomon M, Tenenhouse HS, Jones G. 1990. Expression of 25-hydroxyvitamin D3-24-hydroxylase activity in Caco-2 cells. An in vitro model of intestinal vitamin D catabolism. Endocrinology 126:2868–2875.

Usui E, Noshiro M, Okuda K. 1990. Molecular cloning of cDNA for vitamin D3 25-hydroxylase from rat liver mitochondria. FEBS Lett 262:135–138.

Van Baelen H, Bouillon R, De Moor P. 1980. Vitamin D-binding protein (Gc-globulin) binds actin. J Biol Chem 255:2270–2272.

Vantieghem K, Kissmeyer AM, Haes PD, Bouillon R, Segaert S. 2005. UVB-induced production of 1,25-

dihydroxyvitamin D(3) and vitamin D activity in human keratinocytes pretreated with a sterol Delta(7)-reductase inhibitor. J Cell Biochem epub ahead of print (2005 Dec 13).

Xu D, Fyfe JC. 2000. Cubilin expression and posttranslational modification in the canine gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 279:G748–G756.

Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M. 2001. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 86:888–894.