Abstract. Phytoplankton and zooplankton have been producing vitamin D for more than 500 million years. While the function of vitamin D in the physiology of lower non-vertebrate organisms is not well understood, it is known that most vertebrates need vitamin D to develop and maintain a healthy mineralized skeleton. However, recent findings have demonstrated that 1,25(OH)2D, the biologically-active vitamin D metabolite, exerts a multitude of important physiological effects independently of the regulation of calcium and bone metabolism. These new functions of vitamin D include protection against cancer and other diseases in various tissues. In this review, current knowledge of an additional new function of the cutaneous photosynthesis of vitamin D, that has recently emerged, is summarized: the role of vitamin D as an evolutionary highly-conserved endocrine system that protects the skin and other tissues against environmental hazards, including ionizing and UV-radiation, microbial infections and oxidative stress, is discussed.

For more than 500 million years, phytoplankton and zooplankton have been producing vitamin D (1). While the role of vitamin D in the physiology of lower non-vertebrate organisms is not well understood, it is well known that most vertebrates have to obtain an adequate source of vitamin D in order to develop and maintain a healthy mineralized skeleton (1). Once vitamin D has been absorbed from the diet or synthesized in the skin by the action of sunlight, it is metabolized in the liver to 25-hydroxyvitamin D [25(OH)D] and then in the kidney to 1,25-dihydroxyvitamin D [1,25(OH)2D]. There are two principal enzymes involved in the formation of circulating 1,25(OH)2D from vitamin D, the hepatic microsomal or mitochondrial vitamin D 25-hydroxylase (25OHase) and the renal mitochondrial enzyme 1·-hydroxylase (1·OHase) for vitamin D and 25(OH)D, respectively (2, 3). 1,25(OH)2D is metabolized in target cells at least in part by 24OHase, resulting in a specific C-24 oxidation pathway to yield the biliary excretory product calcitroic acid. These hydroxylases belong to a class of proteins known as the cytochrome P450 mixed function mono-oxidases (2, 3). Interestingly, it has been shown that epidermal keratinocytes and various other cell types, including macrophages, melanocytes, prostate, lung and colon cancer cells, contain the enzymatic machinery needed to produce 1,25(OH)2D from vitamin D, the hepatic microsomal or mitochondrial vitamin D 25-hydroxylase (25OHase) and the renal mitochondrial enzyme 1α-hydroxylase (1αOHase) for vitamin D and 25(OH)D, respectively (2, 3). 1,25(OH)2D is metabolized in target cells at least in part by 24OHase, resulting in a specific C-24 oxidation pathway to yield the biliary excretory product calcitroic acid. These hydroxylases belong to a class of proteins known as the cytochrome P450 mixed function mono-oxidases (2, 3).

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important physiological effects, including protection against cancer and other diseases. Our present knowledge about these important new physiological effects of vitamin D metabolites is growing rapidly. The insights into the new biological functions of 1,25(OH)2D in regulating cell growth, modulating the immune system and modulating the renin-angiotensin system provide an explanation for why reduced sun exposure at higher latitudes is associated with increased risk of dying from many types of cancers, developing type 1 diabetes and multiple sclerosis and having a higher incidence of arterial hypertension. In this review, the current knowledge about another function of vitamin D metabolites, which has only recently been appreciated, is summarized: namely the cutaneous photosynthesis of vitamin D as an evolutionary highly-conserved endocrine system that protects the skin and other organs against environmental hazards, including ionizing and UV-radiation, microbial infections and oxidative stress.

The Vitamin D Endocrine System Protects against UV-radiation

The hazardous effects of solar ultraviolet (UV)-radiation, in particular of UV-B with a wavelength range between 290 and 320 nm, are well recognized as the most important etiological factor in the development of non-melanoma skin cancer (8, 9). UV-B induces photochemical changes in the skin that may lead to acute effects such as sunburn and immune suppression, or chronic effects like premature skin aging and skin cancer (8). Two well-known UV-B-mediated biological effects are the induction of apoptosis (10, 11) and the production of interleukin-6 (IL-6) (12). Apoptosis, as a mode of programmed cell death, is induced following UV-B-irradiation when cellular damage is too severe to be repaired (10, 11). To induce apoptosis, UV-B modulates a variety of important cellular signaling pathways that involve various nuclear and cell surface death receptors and the formation of reactive oxygen species (ROS) (12). Also involved in this process is the activation of a cascade of cysteine proteases called caspasas (12). The final effector protease, caspase 3, causes cleavage of several substrates, including poly(ADP-ribose)polymerase (PARP), which immediately results in apoptosis (12). This cascade appears to be crucial for executing apoptosis induced by UV-B (12). Furthermore, it has recently been described that c-Jun-NH2-terminal kinase (JNK), a member of the mitogen-activated protein kinases (MAPK), is required for UV-induced apoptosis via the induction of cytochrome c release (12). It has been hypothesized that JNK-dependent apoptosis is mediated through mitochondrial cytochrome c release, which has also been observed as an early event in UV-mediated apoptosis in HaCaT cells (12). On the other hand, UV-B-irradiation strongly induces the accumulation of IL-6 mRNA and the release of IL-6 protein by human keratinocytes (12). The cytokine IL-6 is an important mediator of the sunburn reaction and of UV-B-dependent immune suppression (10). Furthermore, IL-6 has been implicated in the tumorigenesis of basal cell carcinoma, a frequent neoplasm that can be induced by UV-B-radiation (10, 11).

It has been shown convincingly that the biologically-active vitamin D metabolite 1,25(OH)2D protects human skin cells from UV-induced cell death and apoptosis (10-12). In these studies, the cytoprotective effects of 1,25(OH)2D on UV-B-irradiated keratinocytes were seen morphologically and by using a colorimetric cell survival assay (12). Moreover, using an ELISA that detects DNA fragmentation, it was shown that pre-treatment with 1,25(OH)2D suppressed UV-B-induced apoptotic cell death (12). Pre-treatment of keratinocytes with 1,25(OH)2D (1 µM) for 24 h reduced UV-B-stimulated apoptosis by 55-70%. This suppression required pharmacological concentrations of 1,25(OH)2D and a pre-incubation period of several hours (12). In addition, it was demonstrated that pre-treatment with 1,25(OH)2D also inhibited mitochondrial cytochrome c release (90%), a hallmark event of UV-B-induced apoptosis (12). Furthermore, it was demonstrated that 1,25(OH)2D reduced two important mediators of the UV-response, namely, JNK activation and IL-6 production (12). As shown by Western blotting, pre-treatment of keratinocytes with 1,25(OH)2D (1 µM) diminished UV-B-stimulated JNK activation by more than 30%. Furthermore, 1,25(OH)2D treatment (1 µM) reduced the UV-B-induced IL-6 mRNA expression and protein secretion by 75-90%. Analyzing the cleavage of PARP further corroborated these observations. As mentioned before, PARP cleavage is clearly induced by UV-B-irradiation. In recent studies, it was shown that pre-treatment of keratinocytes with 1,25(OH)2D (1 µM for 24 h) was able to efficiently, but not completely, inhibit this UV-B-induced PARP cleavage (12). Apart from these effects, metallothionein(MT)-induction may be relevant for the anti-UV-B effects of 1,25(OH)2D. MT acts as a radical scavenger in oxygen-mediated UV-B injury (12). MTs are a class of small cysteine-rich proteins that bind and exchange heavy metal ions, but also have clear scavenging properties for ROS (12). Indeed, part of the UV-B-induced damage to cells occurs through the formation of ROS and antioxidative agents such as MT have been reported to be photoprotective (12). MT mRNA expression was clearly induced by 1,25(OH)2D. Recently, the anti-apoptotic effect of 1,25(OH)2D in keratinocytes was confirmed, using cisplatin and doxorubicin as apoptotic triggers (11). In that study, it was demonstrated that 1,25(OH)2D activated two independent survival pathways in keratinocytes: the MEK/extracellular signal-regulated kinase (ERK) and the phosphatidylinositol 3-kinase (PI-3K)/Akt pathway (11). Activation of ERK and Akt by 1,25(OH)2D was transient, required a minimal dose of 10-9 M and could be blocked by...
The photoprotective effects of 1,25(OH)2D, whilst a genomic 1,25(OH)2D (TX 522) and 19-nor-14,20-bisepi-23-yne-De Haes protective effect against UV-B-induced DNA damage (13). Other investigators showed that the antiproliferative capacity of 1,25(OH)2D underlies its protective effect against UV-B-induced DNA damage (13). De Haes et al. (13) demonstrated that 19-nor-14-epi-23-yne-1,25(OH)2D (TX 522) and 19-nor-14,20-bisepi-23-yne-1,25(OH)2D (TX 527), two low-calcemic analogs of 1,25(OH)2D, were 100 times more potent than the parent molecule in inhibiting UV-B-induced DNA damage (13). It was speculated that these molecules, therefore, may represent promising candidates for the chemoprevention of UV-B-induced skin cancer (13). Other investigators showed that treatment with three different vitamin D compounds (1,25(OH)2D; the rapid acting, low calcemic analog, 1,25(OH)2D protected primary human keratinocytes against the induction of CPDs by UV-B (13). This protection required pharmacological doses of 1,25(OH)2D and an incubation period of at least 8 h before irradiation. It has been speculated that the antiproliferative capacity of 1,25(OH)2D underlies its protective effect against UV-B-induced DNA damage (13). De Haes et al. (13) demonstrated that 19-nor-14-epi-23-yne-1,25(OH)2D (TX 522) and 19-nor-14,20-bisepi-23-yne-1,25(OH)2D (TX 527), two low-calcemic analogs of 1,25(OH)2D, were 100 times more potent than the parent molecule in inhibiting UV-B-induced DNA damage (13). It was speculated that these molecules, therefore, may represent promising candidates for the chemoprevention of UV-B-induced skin cancer (13). Other investigators showed that treatment with three different vitamin D compounds (1,25(OH)2D; the rapid acting, low calcemic analog, 1alpha,25(OH)(2)lumisterol(3) (JN); and the low calcemic but transcriptionally-active hybrid analog 1alpha-hydroxymethyl-16-ene-24,24-difluoro-25-hydroxy-26,27-bis-homovitamin D3 QW-1624F2-2 (QW)) diminished, in all skin cell types, the numbers of UV-induced pre-mutagenic CPDs from 0.5 h after the cessation of UV-radiation, which may explain the enhanced survival of skin cells (14). In that study, the rapid response antagonist analog Ibeta,25(OH)2D3 (HL) abolished the photoprotective effects of 1,25(OH)2D, whilst a genomic antagonist, (23S)-25-dehydro-1alpha-hydroxyvitamin D3-26,23-lactone (TEI-9647), had no effect (14). UV-radiation increased p53 expression in human skin cells and concurrent treatment with 1,25(OH)2D further enhanced this effect several fold, at 3 and 6 h after UV-radiation (14). Combined with previously reported lower nitrite levels in the presence of 1,25(OH)2D, it has been speculated that this increased p53 expression may favor DNA repair over apoptosis (14). Additionally, it was shown convincingly that topical application of 1,25(OH)2D or QW suppressed solar-simulated UV (SSUVR)-induced pyrimidine dimers in the epidermis of irradiated hairless Skh:HR1 mice, measured 24 h after irradiation (14). Furthermore, the UV-induced immuno-suppression in the mice was markedly reduced by topical application of either 1,25(OH)2D or QW (14). Taking these data together, a protective effect of vitamin D compounds against UV-B-induced photodamage was convincingly shown in vitro and in vivo. It is tempting to speculate that the UV-B-induced cutaneous production of vitamin D may represent an evolutionarily highly-conserved feed-back mechanism that protects the skin from the hazardous effects of solar UV-radiation.

The Vitamin D Endocrine System Protects against Ionizing Radiation

Preliminary laboratory investigations (15) point towards a protective effect of 1,25(OH)2D against ionizing radiation-induced cell damage. Using gene array methodology, it was shown that pre-treatment with 1,25(OH)2D followed by ionizing radiation of the human keratinocyte cell line, HaCaT, resulted in a down-regulation of various apoptosis-relevant genes in a dose-dependent manner (15). These findings indicated that biologically-active vitamin D compounds may protect keratinocytes and other cell types from ionizing radiation-induced apoptosis.

The Vitamin D Endocrine System Protects against Oxidative Stress

It is well known that the activation of the stress-activated protein kinases (SAPKs), such as JNK and p38, is an early cellular response to stress signals and an important determinant of cell fate. It has been demonstrated that modulation of these SAPKs is associated with the effects of 1,25(OH)2D on keratinocytes under stress. When HaCaT keratinocytes were exposed to heat shock, hyperosmotic concentrations of sorbitol, the epidermal growth factor receptor tyrosine kinase inhibitor AG1487, the pro-inflammatory cytokine tumor necrosis factor alpha and H2O2, these stress factors activated both SAPKs (16). Pretreatment with 1,25(OH)2D inhibited the activation of JNK by all stresses and the activation of p38 by heat shock, AG1478 and tumor necrosis factor alpha (16). Under the same conditions, treatment with 1,25(OH)2D protected HaCaT keratinocytes from the cytotoxicity induced by exposure to H2O2 and hyperosmotic shock (16). In that study, the effect of 1,25(OH)2D was dose-dependent, already apparent at nanomolar concentrations and time-dependent,
with maximum effects after a 24-h pre-incubation. It has recently been suggested that the inhibition of SAPK activation may account for some of the well-documented protective effects of 1,25(OH)_{2}D on epidermal cells during exposure to UV or chemotherapy and may also be related to the anti-inflammatory actions of the hormone in the skin.

Interestingly, it has been demonstrated that 1,25(OH)_{2}D inhibited caspase-3-like activation in HaCaT keratinocytes exposed to hyperosmotic and oxidative stresses, heat shock and the inflammatory cytokine TNF (17). In that study, it was shown that the hormone also protected the cells from caspase-independent cell death induced by hyperosmotic and oxidative stresses. The protection against hyperosmotic stress was not affected by inhibitors of the EGF receptor, ERK or PI13 kinase pathways, neither was it due to reduced activity of the pro-apoptotic p38 MAP kinase. In conclusion, these results are in accordance with previous in vivo findings that vitamin D protects epidermal keratinocytes from apoptosis due to UV-radiation or chemotherapy, as discussed earlier in this review.

The Vitamin D Endocrine System Protects against Infectious Agents

Recently, it was shown that 1,25(OH)_{2}D is a direct regulator of antimicrobial innate immune responses (18-20). The innate immune system of mammals provides a rapid response to repel assaults from numerous infectious agents including bacteria, viruses, fungi and parasites. A major constituent of this system is an array of cationic antimicrobial peptides (AMP) that include the α- and β-defensins and cathelicidins. Because bacteria have difficulty in developing resistance to AMPs and are quickly killed by them, this class of antimicrobial agents is being commercially developed for clinical use as peptide antibiotics (18). The majority of the pharmaceutical efforts have concentrated on the development of topically-applied agents (18). The expense and difficulty of preparing large amounts of peptides and the uncertainty about possible side-effects in the systemic use of these peptides have slowed their development beyond topical treatments. The cathelicidins are a class of mammalian antimicrobial peptides expressed in leukocytes and on epithelial surfaces (21). Human cathelicidin antimicrobial protein hCAP18 is encoded by CAMP (Gene ID ENS G00000164047) on the chromosomal location 3p21 and is the sole cathelicidin protein in humans detected to date. The cathelicidins are characterized by a C-terminal cationic AMP domain that is activated by cleavage from the N-terminal cathelin portion of the propeptide. The majority of the CAMP propeptide is stored in secondary or specific granules of neutrophils from which it can be released at sites of microbial infection (18). In addition to neutrophils, various white blood cell populations were shown to express hCAP18. These include natural killer cells, T cells, B cells, monocytes and mast cells (18). CAMP is synthesized and secreted in significant amounts by tissues that are exposed to environmental microbes. These include the squamous epithelia of the mouth, tongue, esophagus, lungs, intestine, cervix and vagina (18). In addition, it is produced by salivary and sweat glands, the epididymis, testis and mammary glands (18). Consequently, the polypeptide may be found in wounds, sweat, airway surface fluids, seminal plasma and milk (18). Recent studies have shown that cathelicidins, in addition to being antimicrobial, are multifunctional proteins with receptor-mediated effects on eukaryotic cells and activity in chemotaxis, angiogenesis and wound healing (21, 22). In the skin, there is a low constitutive expression of hCAP18 in the basal epidermal layer, but rapid up-regulation upon inflammation and injury (23-25). The possibility of extrinsically manipulating the endogenous expression of CAMP for systemic and localized therapeutic benefits is very attractive. Since their discovery more than a decade ago, the majority of expression studies have focused on the detection of cathelicidins in various tissues; however, the transcriptional mechanisms that regulate cathelicidin gene expression have not been adequately elucidated. Because AMPs serve a role in the host defense and may act as mediators of other biological processes, their expression is tightly regulated. The molecular mechanisms controlling the expression of CAMP are still poorly understood. Interestingly, the promoters of the human CAMP and defensin 2 (defB2) genes contain consensus VDRE that mediate 1,25(OH)_{2}D-dependent gene expression (19). 1,25(OH)_{2}D induces antimicrobial peptide gene expression in isolated human keratinocytes, monocytes and neutrophils, as well as in various human cell lines, and 1,25(OH)_{2}D along with LPS synergistically induces CAMP expression in neutrophils (19). Moreover, 1,25(OH)_{2}D induces corresponding increases in antimicrobial proteins and the secretion of antimicrobial activity against pathogens including Pseudomonas aeruginosa (19). Weber et al. (20) convincingly demonstrated, in human keratinocytes, an up-regulation of CAMP of about one order of magnitude by treatment with 100 nM 1,25(OH)_{2}D or MC903 (calcipotriol, a vitamin D analog used for psoriasis treatment (26)). In this study, pre-treatment of the cells for 48 h with 1.5 mM calcium, that is known to regulate the major functions of the epidermis including terminal differentiation, increased the expression of CAMP by about 1.5-fold, and was synergistic to the effects of 1,25(OH)_{2}D or MC903. Surprisingly, 25(OH)D_{3}, the precursor of biologically-active 1,25(OH)_{2}D, stimulated CAMP expression to the same magnitude as 1,25(OH)_{2}D or MC903. The vitamin D analogs, corresponding to 25(OH)D_{2} or 1,25(OH)_{2}D, were slightly less efficient. In this study, all the compounds were active
down to levels of 10 nM while the precursor of vitamin D biosynthesis, 7-dehydrocholesterol (7-DHC), was ineffective at all concentrations tested (20). Independent investigations, confirmed by Western blot analysis, that the elevated transcription of CAMP was indeed reflected at the protein level (18, 20). The induction of CAMP expression occurred via a consensus VDRE in the CAMP promoter that was bound by the VDR. The induction of CAMP in murine cells could not be observed and the expression of CAMP mRNA in murine VDR-deficient bone marrow was similar to the wild-type levels (18). A comparison of mammalian genomes revealed evolutionary conservation of the VDRE in a short interspersed nuclear element (SINE) in the CAMP promoter of primates that was absent in mouse, rat and canine genomes (18).

Thus, there is convincing evidence that 1,25(OH)2D and its analogs directly regulate antimicrobial peptide gene expression in humans, revealing the potential of these compounds for the treatment of opportunistic infections.

Conclusion

Taking all the data together, increasing evidence now strongly supports the concept that the cutaneous photosynthesis of vitamin D has an important and evolutionarily highly-conserved function as an endocrine system that protects the skin and other tissues against various environmental hazards, including ionizing and UV-radiation, microbial infections and oxidative stress. It can be speculated that vitamin D compounds may represent promising cytoprotective drugs. However, one has to bear in mind that most of these cytoprotective effects are dose-dependent and that, depending on the dosage, the vitamin D analogs may exert opposite effects on survival following cellular damage.

References


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