Unravelling of hidden secrets
The role of vitamin D in skin aging

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Keywords: vitamin D, 25-hydroxyvitamin D, vitamin D receptor, fountain of youth, skin, aging, skin aging

The skin is the only tissue in the human body that represents both a target tissue for biologically active vitamin D compounds including 1,25-dihydroxyvitamin D [1,25(OH)D] and has the capacity for the synthesis of 1,25(OH)D from 7-dehydrocholesterol (7-DHC). Recent findings indicate that the vitamin D endocrine system (VDES), besides multiple other important functions, regulates aging in many tissues, including skin. This concept is strongly supported by several independent studies in genetically modified mice (including FGF23−/− and Klotho−/− mice) that are characterized by altered mineral homeostasis caused by a high vitamin D activity. These mice typically have phenotypic features of premature aging that include, besides short lifespan, retarded growth, ectopic calcification, immunological deficiency, osteoporosis, atherosclerosis, hypogonadism, skin and general organ atrophy. Notably, it has been demonstrated that these phenotypic features can be reversed by normalizing mineral homeostasis and/or vitamin D status. Interestingly, the aging phenotypes of mice suffering from hypovitaminosis D (VDR−/−) and CYP27B1−/− mice) are quite similar to those suffering from hypervitaminosis D (including FGF-23−/− and Klotho−/− mice). Consequently, it has been hypothesized that thus, both hypovitaminosis D and hypervitaminosis D may enhance aging. Aging seems to show a U-shaped response curve to vitamin D status, and, therefore normovitaminosis D seems to be important for preventing premature aging. Additionally, laboratory investigations have now convincingly shown that vitamin D compounds protect the skin against the hazardous effects of various skin aging-inducing agents, including ultraviolet (UV) radiation. In conclusion, these findings support the concept that UV-radiation exerts both skin aging-promoting and -inhibiting effects, the latter via induction of cutaneous vitamin D synthesis. Future studies will clarify the effect of vitamin D compounds on expression and function of potential key regulators of skin aging, such as TAp63 or the IGF-1 signaling pathway. Furthermore, the efficacy of topically applied vitamin D compounds in the prevention of skin aging has to be evaluated in future clinical trials.

Introduction

In recent years, there was an extensive search for the “fountain of youth,” that means for pharmacologic agents effective in preventing tissue aging. Although the “fountain of youth” has still not been found, there is at present still enormous interest in identifying pharmacologic agents that may prevent skin aging. Recent findings indicate that the vitamin D endocrine system (VDES), besides many other important functions, regulates aging in many tissues, including skin. In this review, I summarize our present understanding of the role of the VDES for skin aging and demonstrate that vitamin D compounds represent interesting candidates for the prevention of skin aging.

The Vitamin D Endocrine System

It is well known that vitamin D, the precursor of the biologically active vitamin D metabolite 1,25-dihydroxyvitamin D [1,25(OH)2D, calcitriol] can be obtained from the diet or synthesized in the skin under the influence of UV-B radiation from 7-dehydrocholesterol (7-DHC). It has been estimated that under present living conditions in most countries in Europe and US, appr. 90% of the needed vitamin D must be synthesized in the skin and only about 10% are taken up by the diet. The skin is the only tissue in the human body that represents both a target tissue for biologically active vitamin D compounds including 1,25(OH)2D3 and has the capacity for the synthesis of 1,25(OH)2D3 from 7-DHC. Several enzymatic reactions are involved in the photochemical cutaneous synthesis of vitamin D, hereunder 4 photo-reversible reactions and one non-reversible phototransformation. While vitamin D2 (ergocalciferol) can be found in plants, vitamin D3 (cholecalciferol) is photochemically synthesized under the influence of UVB radiation in the skin of animals and humans. The biologically active vitamin D metabolite 1,25(OH)2D3, that circulates in the blood, is synthesized from vitamin D by a well characterized biochemical reaction cascade. First, it is hydroxylated in the liver in C-25 position by cytochrome P450 enzymes, including the vitamin D-25-hydroxylase (CYP27A1) and CYP2R1, before it gets hydroxylated a second time in the kidney in C-1 position by another cytochrome P450 enzyme, the renal 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1). The production of 1,25(OH)2D3 in the kidney is regulated by a feedback-mechanism of the hormone itself, as well as by parathyroid hormone, calcium and cytokines like Interferon γ.
(IFNγ) or tumor necrosis factor α (TNFα). Until the last decades of the last century, it was concluded that the kidney was the sole source of 1,25(OH)₂D₃ production. However, more recent investigations demonstrated that many cell types including human keratinocytes, monocytes, macrophages osteoplasts, prostate and colon cells, express the enzymatic machinery for the synthesis of 1,25(OH)₂D₃ (i.e., CYP27B1), and are able to synthesize 1,25(OH)₂D₂. In keratinocytes, studies could prove the presence of 1α-hydroxylase (CYP27A1) and 25-hydroxylase (CYP27B1). According to these findings, the keratinocyte is the only cell type known today, that is able to synthesize 1,25(OH)₂D₃ from 7-DHC.

The metabolism of 1,25(OH)₂D₃ circulating in the blood and present in various tissues, to calcitriol results from another well-characterized biochemical reaction cascade. The reduction of 1,25(OH)₂D₃ to 24,25-dihydroxycholecalciferol in the kidney (and in other tissues) and consecutive reactions are effectuated by another cytochrome P450 enzyme, the 1,25-dihydroxyvitamin D₃-24-hydroxylase (CYP24A1). 24,25-dihydroxycholecalciferol has only a slight biological activity. According to present knowledge 1,25(OH)₂D₃ has a 100 to 1000-fold higher biological activity as compared with other natural vitamin D metabolites.

1,25(OH)₂D₃ exerts at least many of its biologic effects via binding to the nuclear vitamin D receptor (VDR). VDR is a member of the thyroid hormone and retinoic acid receptor subfamily of nuclear hormone receptors that bind to specific response elements in target genes following heterodimerization with retinoid X receptor (RXR) isomers. VDR regulates the expression of genes encoding factors which control important cellular functions implicated in skin aging, including proliferation, differentiation, metabolism, ion transport, apoptosis, and detoxification.

**Vitamin D Compounds Protect the Skin Against the Hazardous Effects of Various Skin Aging-Inducing Agents, Including Ultraviolet (UV) Radiation**

It is well accepted in the scientific community that vitamin D compounds protect the skin against the hazardous effects of many skin aging-inducing agents, including ultraviolet (UV) radiation. Concerning protection against UV-B-induced cellular damage, the underlying mechanisms are manifold and at least in part associated with the suppression of UV-B-induced apoptosis. However, it has to be noted that it depends on many other factors whether effects of 1,25(OH)₂D₃ on UV-induced apoptosis are beneficial or negative for the human body. In vitro investigations using an ELISA that detects DNA-fragmentation, it was demonstrated that pretreatment of keratinocytes with 1,25(OH)₂D₃ (1 μM) over 24 h suppresses UV-B-induced apoptosis up to 55–70%, and reduces the mitochondrial cytochrome c release, one of the markers of UV-B-induced apoptosis, up to 90%. Two important mediators of the UV-response in keratinocytes, namely the activation of a Jun-NH₂-terminal kinase and the production of interleukin-6, are also reduced about 30% respective 75–90% by a pretreatment with 1,25(OH)₂D₃ (1 μM). Moreover, the UV-B-induced cleavage of the Poly-(ADP-Ribose)-Polymerase (PARP) is inhibited, and methallothionein (MT)-mRNA is induced after pretreatment of keratinocytes with 1,25(OH)₂D₃ (1 μM for 24 h). MT is an antioxidant and acts as a radical catcher after UV-radiation. This may represent an important mechanism, protecting keratinocytes and other cells against the UV-B-induced synthesis of reactive oxygen radicals.

Moreover, 1,25(OH)₂D₃ protects skin cells against apoptosis by induction of many anti-apoptotic proteins including Bcl-2 and activation of the MEK/ERK- and PI-3K/Akt-metabolic pathways. 1,25(OH)₂D₃ is able to induce the neutral Mg²⁺-dependent sphingomyelinase, which hydrolyses sphingomyelin to ceramide. Interestingly, ceramide stimulates the prodifferentiating effect from 1,25(OH)₂D₃ on keratinocytes. Moreover, it plays an important role in the induction of apoptosis in a variety of cells, including keratinocytes. It has been demonstrated that physiological concentrations of 1,25(OH)₂D₃ in keratinocyte cultures do not induce apoptosis. To the contrary, physiological concentrations of 1,25(OH)₂D₃ generate an apoptosis-resistance against ceramides, UV radiation and TNFα. The cytoprotective/anti-apoptotic effect of 1,25(OH)₂D₃ is obviously linked to the development of sphingosine-1-phosphate. This is also clarified by the fact that the antiapoptotic effect of 1,25(OH)₂D₃ can be completely suppressed by addition of the sphingosinokinase-inhibitor N,N-dimethylsphingosine. In contrast, pharmacological concentrations of 1,25(OH)₂D₃ (≥10⁻⁸ M) do induce apoptosis. Similar effects have been observed in the regulation of keratinocyte growth, where, as outlined above, physiological concentrations of 1,25(OH)₂D₃ (around 10⁻¹¹ M) stimulate cell proliferation, whereas high pharmacological concentrations of 1,25(OH)₂D₃ have a dose-dependent antiproliferative effect.

In line with these findings, we have recently used colony-forming-unit culture proliferation assays to prove that pretreatment with 1,25(OH)₂D₃ (10⁻⁷ M for 48 h) protects human keratinocytes against the hazardous effects of a single irradiation with 100 J/cm² UV-B. In this study, the number of cell colonies counted after a growth period for 7 d post radiation was twice as high in 1,25(OH)₂D₃ pretreated cells as compared with controls that were not treated with 1,25(OH)₂D₃. Furthermore, using WST-1- and crystal violet-based proliferation assays, it could be demonstrated that 1,25(OH)₂D₃ (10⁻⁷ M) has a protective effect after irradiation of keratinocytes with ascending doses of UV-B (100–1000 J/cm²). It is well recognized that the photocarcinogenesis of non-melanoma skin cancer is mainly due to mutations resulting from insufficient repaired DNA-photoproducts. The most established DNA-photoproducts caused by UV radiation are cyclobutane pyrimidine dimers (CPDs). Recent laboratory investigations show that treatment with 1,25(OH)₂D₃ reduces the number of CPDs in human keratinocytes after UV-B-radiation. A study from Gupta et al. describes a reduction of the number of CPDs after a pretreatment with 1,25(OH)₂D₃ (10⁻⁷ M) followed by an irradiation of the cells with 200 mJ/cm² UV-B, as compared with controls that were not treated with 1,25(OH)₂D₃. In line with these results, Trémezaygues et al. demonstrated that pretreatment of keratinocytes with 1,25(OH)₂D₃ (10⁻⁷ M) has a protective effect on the cells, even after irradiation with higher doses of UV-B (100 J/cm² and 1000 J/cm²). Complementing the results of Gupta et al., Trémezaygues et al.
showed that pretreatment of HaCaT-keratinocytes with 1,25(OH)\(_2\)D results not only in a reduction of the number of formed CPDs but also in a subsequent quicker reduction in number of CPDs, as compared with controls that were not treated with 1,25(OH)\(_2\)D.\(^9\)

Regarding the influence of vitamin D metabolites on the development of ionizing radiation damage, several investigations were performed during the last years.\(^9\) A characteristic feature of cellular damage induced by ionizing radiation are DNA double strand breaks (DSB). The histone protein H2AX is phosphorylated in position 139 at the carboxyterminus as an answer to a double strand break—the result is γH2AX. This phosphorylated histone protein supports a recruitment and retention of various repair proteins at the site of the DSB. It can therefore be considered as a marker for DSBs. Recent studies show a decreased immunoreactivity for γH2AX caused by ionizing radiation after pretreatment of cells with 1,25(OH)\(_2\)D (10\(^{-7}\) M).\(^9\)

Interestingly, it has been demonstrated that photoprotection by 1,25-dihydroxyvitamin D is associated by an increase in p53 and a decrease in nitric oxide products.\(^18\) Both members of the p53 family and nitric oxide have been implicated to be of importance for skin aging.\(^19-21\)

To put it in a nutshell, the current literature convincingly supports the concept of a cytoprotective effect of 1,25(OH)\(_2\)D against the damaging effects of UV and other agents, which may help to prevent premature skin aging. The clinical potential of this protective effect has to be elucidated in future clinical trials.

**Vitamin D Status and Skin Aging: Animal Studies in Genetically Modified Mice Provide Evidence For a U-Shaped Curve**

The concept that the VDES, besides many other important functions, regulates aging in skin and many other tissues, is strongly supported by several independent studies in genetically modified mice (including FGF-23\(^-/-\) and Klotho\(^-/-\) mice that develop altered mineral homeostasis caused by a high vitamin D activity).\(^22-25\) These mice typically have phenotypic features of premature aging that include, besides short lifespan, skin and general organ atrophy, retarded growth, ectopic calcification, immunological deficiency, osteoporosis, atherosclerosis, and hypogonadism.\(^22-25\) Notably, it has been demonstrated that these phenotypic features can be reversed by normalizing mineral homeostasis (by a rescue diet containing high calcium and phosphate) and/or vitamin D.\(^22-25\) Interestingly, the aging phenotypes of mice suffering from hypovitaminosis D (VDR\(^-/-\) and CYP27B1\(^-/-\) mice) are quite similar to those suffering from hypervitaminosis D (including FGF-23\(^-/-\) and Klotho\(^-/-\) mice).\(^22,24\) VDR\(^-/-\) mice are characterized by skin thickening and wrinkling, alopecia, have growth retardation, osteoporosis, kyphosis, ectopic calcification, progressive loss of hearing and balance as well as a relatively short lifespan.\(^22,24\) Consequently, it has been hypothesized that thus, both hypov- and hypervitaminosis D may enhance aging and that aging seems to show a U-shaped response curve to vitamin D status, and, therefore normovitaminosis D seems to be important for preventing premature aging.\(^22,24\)

**Vitamin D Status and Skin Aging: What Are the Molecular Mechanisms by Which Vitamin D Signaling Feeds the “Fountain of Youth”?**

It has to be noted that at present, the molecular mechanisms that underly the anti-aging effects of vitamin D compounds are not well understood. Based upon many investigations, including cDNA microarray analysis of mRNAs, as many as 500–1000 genes are estimated to be regulated by VDR ligands.\(^25\) Many of these 1,25(OH)\(_2\)D-regulated genes that are relevant for healthy aging of the skin and other tissues are calcemic, phosphatemic, or affect bone remodelling.\(^25\) It has convincingly been outlined in a review by Mark Haussler et al. that the VDES controls the expression of at least 11 genes (osteopontin or SPPI, TRPV6, LRP5, BGP, RANKL, OPG, CYP24A1, PTH, FGF-23, PHEX, and klotho) which encode bone and mineral homeostasis effectors that also facilitate aging well.\(^25\) To govern these 1,25(OH)\(_2\)D-induced phenomena, there exists a separate class of feedback regulatory genes which curb the mineralotropic and osteotropic actions of 1,25(OH)\(_2\)D.\(^25\) Control of these genes by VDR delimits bone mineralization to the defined endoskeleton, prevents ectopic calcification elicited by excesses of either calcium or phosphate, reduces age-related vascular pathology and atherosclerosis, protects against muscle and skin atrophy as well as respiratory failure, and generally prevents premature aging and lengthens lifespan.\(^23,25\) Many of these pathologies are also the result of hypervitaminosis D.\(^23,25\) Consequently, it has been hypothesized that excess vitamin D and its actions actually may reduce lifespan, meaning that the level of 1,25(OH)\(_2\)D as well as the sequelae of its effects through VDR must be “detoxified” and sustained in an optimal range to maintain healthful aging.\(^23,25\)

Interestingly, the association of vitamin D-deficiency with some types of cancer has been convincingly demonstrated and both aging and cancer are promoted by some similar molecular mechanisms.\(^27\) As an example, damage on DNA and telomeres cause both aging and cancer, involving the tumor suppressor protein, p53. Moreover, the insulin-like growth factor (IGF-1) and FGF-23 signaling pathways regulate growth, aging and cancer. Interestingly, the VDES has been shown to regulate these important signaling pathways. Mutations in insulin/IGF-1 signaling pathway have been shown to lead to increased longevity in various invertebrate models, although it has to be noted that it was recently shown that the Igf1r\(^(-/-)\) mouse is not a model of increased longevity and delayed aging as predicted by invertebrate models with mutations in the insulin/IGF-1 signaling pathway.\(^28\)

The p53 gene family, NFκB and telomerase reverse transcriptase (TERT) might be important molecular targets mediating vitamin D action in aging and cancer.\(^29,30\) Since the discovery of the Tp63 gene in 1998, many studies have demonstrated that ΔNp63, a p63 isoform of the p53 gene family, is involved in multiple functions during skin development and in adult stem/progenitor cell regulation.\(^29,30\) Interestingly, a recent investigation demonstrated novel functions for TAp63 indicating a protective role of TAp63 on premature aging.\(^29-30\) TAp63 controls skin homeostasis by maintaining dermal and epidermal progenitor/stem cell pool and protecting them from senescence, DNA damage and genomic instability.\(^29,30\)
Recently, a TAp63 conditional knockout mouse was developed and used to ablate TAP63 in the germline (TAp63−/−) or in K14-expressing cells in the basal layer of the epidermis (TAp63ΔK14Δ). Interestingly, TAp63ΔK14Δ mice age prematurely and develop blisters, skin ulcerations, senescence of hair follicle-associated dermal and epidermal cells, and decreased hair morphogenesis. These data indicate that TAp63 maintains adult skin stem cells by regulating cellular senescence and genomic stability, thereby preventing premature skin aging.

Another interesting observation that may be of high importance for skin aging is the link of the VDES to detoxification. Evolutionarily, the VDR is closely related to the pregnane X receptor (PXR) that modulates xenobiotic detoxification and to the farnesoid X receptor (FXR) which regulates bile acid metabolism. In line with this finding, the ancient function of VDR in chordates is considered to be that of detoxification. This property has apparently been retained in extant mammals as evidenced by the ability of the VDR to bind carcinogenic secondary bile acid, lithocholic acid (LCA), with low affinity and stimulate its detoxification in colon via induction of CYP3A4 and SULT2. It can be speculated whether VDR-induced detoxification may represent an important mechanism to prevent aging in various tissues, including skin.

Conclusions

It can be summarized that the VDES influences skin aging via a broad variety of different mechanisms, that include protection against UV-induced cellular damage, detoxification, and regulation of genes important for cellular aging. The efficacy of topically applied vitamin D compounds and of a healthy vitamin D status for the prevention of skin aging has to be evaluated in future clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266-81; PMID:17634462; http://dx.doi.org/10.1056/NEJMoa070553.

2. Reichrath J. The challenge resulting from positive and negative effects of sunlight: how much solar UV exposure is appropriate to balance risks of vitamin D deficiency and skin cancer? Prog Biophys Mol Biol 2016; 129:9-16; PMID:26603232; http://dx.doi.org/10.1016/j.pbiomolbiol.2016.02.010.

3. Reichrath J. Vitamin D and the skin: an ancient conserved endocrine system that protects against environmental hazards including UV-radiation and microbial infections. Annu Cancer Res 2006; 26(4S):2743-4; PMID:16866866.

4. Lee JH, Youn JI. The photoprotective effect of 1,25-dihydroxyvitamin D3 on ultraviolet B-induced damage in keratinocytes and in a mechanism of action. J Dermatol Sci 1998; 18:11-8; PMID:9574765; http://dx.doi.org/10.1016/S0923-1811(98)00015-2.

5. De Haes P, Garmyn M, Verstuyf A, De Clercq P, De Schrijver A, van den Berghe G, Trémezaygues L, Sticherling M, Pföhler C, Friedrich Re, Reichrath J. Vitamin D and aging: the challenge resulting from positive and negative effects of sunlight: how much solar UV exposure is appropriate to balance risks of vitamin D deficiency and skin cancer? Prog Biophys Mol Biol 2010; 121:164-8; PMID:20399269; http://dx.doi.org/10.1016/j.pbiomolbiol.2010.03.082.

6. Dixon KM, Norman AW, Sequeira VB, Mohan R, Manggau Manggau, Kim DS, Ruwisch L, Vogler R, Korting HC, Schäfer-Korting M, et al. 1alpha,25-Dihydroxyvitamin D3 analogues protect primary human keratinocytes against UVB-induced damage: In vitro analysis of cell viability/proliferation, DNA damage and repair. Dermatoendocrinol 2010; 3:301-8; PMID:20259279; http://dx.doi.org/10.4161/dem.1.1.47097.

7. Gupta R, Dixon KM, Deo SS, Holliday CJ, Slater M, Halliday GM, et al. Photoprotection by 1alpha,25-dihydroxyvitamin D and analogs: further studies on mechanisms and implications for UV-damage. J Steroid Biochem Mol Biol 2007; 109:117-22; PMID:17170736; http://dx.doi.org/10.1016/j.jsbmb.2007.04.003.

8. Holowatz LA, Houghton BL, Wong BJ, Wilkins BW, Harding AW, Kenney WL, et al. Nitric oxide and attenuated reflex cutaneous vasodilation in aged skin. Am J Physiol Heart Circ Physiol 2003; 285:H1662-7; PMID:12505876.

9. Su X, Paris M, Gi YJ, Tsai KY, Cho MS, Lin YL, et al. TAp63 prevents premature aging by promoting adult stem cell maintenance. Cell Stem Cell 2009; 5:64-75; PMID:19570515; http://dx.doi.org/10.1016/j.stem.2009.04.003.

10. Bokov AF, Garg N, Ikeno Y, Thakur S, Musi N, DeFronzo RA, et al. Does reduced IGF-1R signaling in Igf1r−/− mice alter aging? PLoS One 2011; 6:e26891; PMID:22132081; http://dx.doi.org/10.1371/journal.pone.0026891.

11. Lin R, Nagai Y, Sladek R, Bastien Y, Ho J, Petrecca K, et al. Expression profiling in squamous carcinoma cells reveals pleiotropic effects of vitamin D3 analog EB1089 signaling on cell proliferation, differentiation, and immune system regulation. Mol Endocrinol 2007; 21:1243-56; PMID:17044937; http://dx.doi.org/10.1210/me.2006-1243.

12. Haes De, P, Garmyn M, Verstuyf A, De Clercq P, Vandenbever V, Vandenkerckhove K, Vanhove V, et al. Two 14-epi analogues of 1,25-dihydroxyvitamin D3 protect human keratinocytes against the effects of UVB. Arch Dermatol Res 2004; 296:527-34; PMID:15402383; http://dx.doi.org/10.1007/s00403-004-0451-x.

13. De Haes P, Garmyn M, Verstuyf A, De Clercq P, Vandewalle M, Vastenberg K, et al. Two 14-epi analogues of 1,25-dihydroxyvitamin D3 protect human keratinocytes against the effects of UVB. Arch Dermatol Res 2004; 296:527-34; PMID:15402383; http://dx.doi.org/10.1007/s00403-004-0451-x.

14. De Haes P, Garmyn M, Verstuyf A, De Clercq P, Vandewalle M, Deeghert H, et al. 1,25(OH)2D3 and analogues protect primary human keratinocytes against UVB-induced DNA damage. J Photochem Photobiol B: Biol 2005; 78:141-8; PMID:16000187; http://dx.doi.org/10.1016/j.jphotobiol.2004.09.010.

15. Trémezaygues L, Seifert M, Tülg K, Reichrath J. 1,25-dihydroxyvitamin D3 protects human keratinocytes against UV-B-induced damage: In vitro analysis of cell viability/proliferation, DNA damage and repair. Dermatoendocrinol 2009; 1:239-45; PMID:20259279; http://dx.doi.org/10.4161/dem.1.4.70597.