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Review

1α,25-Dihydroxyvitamin D_3 reduces several types of UV-induced DNA damage and contributes to photoprotection

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ABSTRACT

Vitamin D production requires UVB. In turn, we have shown that vitamin D compounds reduce UV-induced damage, including inflammation, sunburn, thymine dimers, the most frequent type of cyclobutane pyrimidine dimer, immunosuppression, and photocarcinogenesis. Our previous studies have shown most of the photoprotective effects by 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) occurred through the nongenomic pathway because similar protection was seen with an analog, 1α,25-dihydroxylumistrol$_3$ (JN), which has little ability to alter gene expression and also because a nongenomic antagonist of $1,25(OH)_2D_3$ abolished protection. In the current study, we tested whether this photoprotective effect would extend to other types of DNA damage, and whether this could be demonstrated in human ex vivo skin, as this model would be suited to pre-clinical testing of topical formulations for photoprotection. In particular, using skin explants, we examined a time course for thymine dimers (TDs), the most abundant DNA photolesion, as well as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), which is a mutagenic DNA base lesion arising from UV-induced oxidative stress, and 8-nitroguanosine (8-NG). Nitric oxide products, known markers for chronic inflammation and carcinogenesis, are also induced by UV. This study showed that $1,25(OH)_2D_3$ significantly reduced TD and 8-NG as early as 30 min post UV, and 8-oxodG at 3 h post UV, confirming the photoprotective effect of $1,25(OH)_2D_3$ against DNA photoproducts in human skin explants. At least in part, the mechanism of photoprotection by $1,25(OH)_2D_3$ is likely to be through the reduction of reactive nitrogen species and the subsequent reduction in oxidative and nitrosative damage.

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Contents

Abbreviations: UVR, ultraviolet radiation; $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; NO, nitric oxide; CPD, cyclobutane pyrimidine dimer; ROS, reactive oxygen species; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; RNS, reactive nitrogen species; 8-NG, 8-nitroguanosine; PBS, phosphate buffered saline.

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1. Introduction

Ultraviolet radiation (UVR) is necessary for the conversion of 7-dehydrocholesterol in keratinocytes to pre-vitamin D, which converts at body temperature to vitamin D. Vitamin D in turn undergoes further hydroxylations to 25-hydroxyvitamin D and then to 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active form of vitamin D, produced mainly in the kidney, but also produced locally in many tissues, including the skin [1,2]. The importance of vitamin D for human health is increasingly recognized not only for its role in protection against musculoskeletal diseases such as osteoporosis and rickets, but also other systemic diseases [3]. The UVB that produces vitamin D has harmful effects when excessive, since it causes deleterious effects in skin and is responsible for more than 90% of all skin cancers through mechanisms already well documented, which include inflammation [4], DNA damage [5–7], mutagenesis [8] and immune suppression [9], all of which lead to photocarcinogenesis.

Vitamin D compounds have been shown to also protect skin against the harmful effects of UV [10–19]. Previous studies by our group have demonstrated the protective effects of $1,25(OH)_2D_3$ against thymine dimers, a major form of photolesion, when administered immediately after irradiation to human skin cells in culture [10,11,14,17,20] and in human [15] and mouse skin [14,16,20]. Apart from thymine dimers, which are the commonest form of cyclobutane pyrimidine dimer (CPD) found in human skin after UV exposure [5,21], UV is also responsible for other types of DNA damage which arise from various oxidative stresses produced by reactive oxygen species (ROS). Another common UV-induced DNA lesion is caused by oxidation of the guanine base by ROS to form 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a promutagenic base byproduct [9,22,23]. The photoproduct 8-oxodG is repaired through base excision repair (BER), which is mediated by the DNA repair enzyme, 8-oxoguanine DNA glycosylase-1 (OGG1) [24]. Accumulation of unrepaired 8-oxodG is known to cause mutations that lead to carcinogenesis, as shown by the increased skin cancer susceptibility in OGG1 knockout mice that have a deficiency in base excision repair [23]. OGG1 was found to be downregulated in basal cell carcinoma [24]. Reactive nitrogen species (RNS) are yet another group of photoproducts that can damage DNA. These are formed by excess levels of nitric oxide (NO) production arising from UV upregulation of NO synthases [25] and also from the release of NO by UVA decomposition of endogenous NO stores [26,27]. Excess NO can combine with ROS to form more genotoxic NO derivatives such as peroxynitrite ($OONO^-$). Peroxynitrite causes base modifications to DNA by oxidation or nitration that can lead to mutagenesis and carcinogenesis [28]. Nitrotyrosine and 8-nitroguanosine (8-NG) are products of nitration of tyrosine in proteins and guanine in DNA, and are considered the markers of inflammation and carcinogenesis [9].

Although $1,25(OH)_2D_3$ is not an antioxidant, we previously reported that after UVR, treatment with this compound reduced nitrite and nitrotyrosine [14,20]. We therefore tested whether topical $1,25(OH)_2D_3$ might reduce not only thymine dimers after UV, but also 8-oxodG and 8-NG in a human ex vivo skin model.

2. Materials and methods

2.1. Ex vivo skin

The collection and handling of human ex vivo skin was approved by the Human Research Ethics Committee of the Royal Prince Alfred Hospital (Camperdown, NSW 2005), and the University of Sydney (Sydney, NSW 2006). Human ex vivo skin was collected from consenting patients undergoing elective surgery. Cold sterile phosphate buffered saline (PBS) was used to transport skin. Skin was processed as soon as possible and within 4 h of surgery. Subcutaneous fat, blood vessels and debris were trimmed off to leave epidermis and dermis only for the study. Skin pieces were dissected into 4 mm pieces with a punch biopsy prior to UV irradiation to minimize delay. Three pieces of skin were prepared and analysed per time point per treatment of UV irradiated and sham irradiated samples in 3 independent experiments, using skin from 3 different donors.

2.2. UV irradiation

The UV source used was an Oriel 1000 W xenon-arc lamp solar simulator (Oriel, Stratford, CT, USA) with an atmospheric attenuation filter (Oriel,) to eliminate UVC (<290 nm). It was pre-calibrated using an OL754 spectroradiometer (Optonics Laboratories Inc., Orlando, FL) and IL1350 broadband radiometer (International Light, Newburyport, MA, USA). A calculated total dose of 4000 mJ/cm^2 (326 mJ/cm^2 UVB (8.15%); 3674 mJ/cm^2 UVA) was delivered to skin samples incubated in sterile Martinez solution (145 mM NaCl, 5.5 mM KCl, 1.2 mM $MgCl_2(H_2O)_6$, 1.2 mM $NaH_2PO_4(H_2O)_2$, 7.5 mM NaHEPES, 7.5 mM HEPES, 10 mM D-glucose and 1 mM $CaCl_2(H_2O)_2$), in a volume that was just enough to surround the tissue without submersion. Spectroradiometer measurements of this simulated solar simulator showed comparable spectral characteristics to the UV spectrum from the sun [29]. The UV dose delivered to ex vivo skin in this study is equivalent to around 5–10 min of exposure to the midday sun in a summer day in Australia, and is around 15% of total UV exposure from a typical tanning session in a tanning facility in USA [30]. Immediately after UV irradiation, skins were treated with vehicle (0.01% ethanol), or 1 nM $1,25(OH)_2D_3$ and incubated in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin and 250 ng/ml amphotericin-B (Sigma–Aldrich, NSW, Australia). This concentration of $1,25(OH)_2D_3$ has previously been shown to produce near maximal protection from UV-induced thymine dimers [14]. Sham-irradiated skin samples were subjected to similar procedures but not irradiated. Skin samples were snap frozen in OCT compound at time points of 0.5, 1, 3, 6, 24 and 48 h and stored at −80 °C. Cryosections (6 μm thickness) were air dried at room temperature for 20 min, fixed in cold acetone at 20 °C for 10 min, and air dried at room temperature for another 20 min and then stored at −80 °C until staining.

2.3. Immunohistochemistry

For detection of thymine dimers, 8-oxodG, and 8-nitroguanosine, sectioned skin was rinsed and rehydrated in