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Immune-modifying properties of topical vitamin D: Focus on dendritic cells and T cells☆

Shelley Gorman*, Melinda A. Judge, Prue H. Hart

Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, West Perth, Western Australia, Australia

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ABSTRACT

Topical creams containing the active form of vitamin D (1,25-dihydroxyvitamin D_3; $1,25(OH)_2D_3$) or analogues of this compound are currently used with some success to treat skin conditions including psoriasis and vitiligo. As well as targeting inflammatory processes in the skin, topical application of $1,25(OH)_2D_3$ also affects the function of immune cells in the skin and draining lymph nodes. Topically applied $1,25(OH)_2D_3$ reduces the number of dendritic cells in the skin, resulting in suppressed immunity and in particular reduced contact hypersensitivity (CHS) responses. Topical $1,25(OH)_2D_3$ may also promote the migration of dendritic cells from the skin to the draining lymph nodes. Skin application of $1,25(OH)_2D_3$ prevented the inflammatory effects of UVB irradiation on lymph node hypertrophy, when cell numbers were examined 4 days after skin treatment. In contrast, when $1,25(OH)_2D_3$ was applied to UVB irradiated skin, there was no reversal in the suppression of CHS responses caused by UVB irradiation. Instead, $1,25(OH)_2D_3$ had an additive effect with UVB to suppress CHS responses to a greater degree than UVB alone. In these studies, $1,25(OH)_2D_3$ was applied to the treated skin of BALB/c mice immediately following UVB irradiation. Finally, topical $1,25(OH)_2D_3$ also enhanced the number and suppressive activity of CD4+CD25+ regulatory T cells in the lymphatic tissue draining skin.

1. Introduction

Vitamin D is an essential hormone synthesised in the skin following the exposure of skin to the UVB wavelengths present in sunlight. The active form of vitamin D, calcitriol (1,25-dihydroxyvitamin D_3, $1,25(OH)_2D_3$), and analogues of this hormone (e.g. calcipotriol, calcipotriene) are successful treatment options for patients with skin diseases such as psoriasis and vitiligo [reviewed by [1]]. Currently, vitamin D is administered topically as a cream directly to diseased skin. The mode by which vitamin D reduces the morbidity of these diseases may occur in part by inhibiting the proliferation and inducing the differentiation of keratinocytes in psoriatic lesions [reviewed by [1]]. An alternate pathway whereby topical vitamin D reduces skin inflammation is by regulating cells of the immune system, which reside both in the skin, and skin-draining lymph nodes (SDLN). The article reviews the reported effects of topical $1,25(OH)_2D_3$ on immune cells, concentrating on responses by the adaptive arm of the immune response and in particular dendritic cells (DC) and T cells.

2. Topical vitamin D regulates immune cell infiltration into psoriatic lesions

Topical calcitriol can modify the accumulation of immune cells within psoriatic plaques. Twice-daily treatment with calcitriol for 4 weeks reduced the proportion of T cells and neutrophils in both the dermis and epidermis of 10 patients with psoriasis [2]. These observations conflict with a report that human T cells treated with $1,25(OH)_2D_3$ (10 nM) *in vitro* upregulate the chemokine receptor CCR10 and have an enhanced capacity to migrate towards the skin-trophic chemokine CCL27 [3]. $1,25(OH)_2D_3$ (~40 nM) treatment *in vitro* also reduced the ability of murine CD4+ T cells to migrate towards stromal cell-derived factor-1 (CXCL12) [4].

3. Regulation of skin-derived dendritic cells by topical vitamin D

Topical $1,25(OH)_2D_3$ regulates skin-derived DC such as Langerhans cells. Skin DC density and phenotype was assessed in the ear skin of BALB/c mice, which were administered $1,25(OH)_2D_3$ (400 ng/day) once a day for 7 days [5]. The dendritic morphol-

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* Corresponding author at: Telethon Institute for Child Health Research, Centre for Child Health Research, the University of Western Australia, PO Box 855, West Perth, Western Australia, 6872, Australia. Tel.: +61 8 9489 7884; fax: +61 8 9489 7700.
E-mail address: shelleyg@ichr.uwa.edu.au (S. Gorman).

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ogy of MHC classII+ cells (putative skin DC) was reduced by $1,25(OH)_2D_3$ with cells becoming rounded 24 h after the final skin treatment. Treatment of ear skin with $1,25(OH)_2D_3$ significantly reduced the number of MHC classII+ cells at that site (by 50%) [5]. These observations have recently been repeated following the chronic treatment of mouse skin with topical calcipotriol [6]. Application of calcipotriol cream to normal human skin for 4 days resulted in a dose-dependent decrease in the number of CD1a+ cells with a dendritic morphology and in the number of dendrites per cell [7].

The reduction in skin DC numbers following topical $1,25(OH)_2D_3$ may have been due to apoptosis of the cells, and/or their migration into the SDLN. In preliminary studies, we have observed significant increases (by a mean of $27 \pm 15\%$, mean $\pm$ SEM, $n = 4$ experiments) in the numbers of skin-derived DC ($CD11c+MHCII+DEC\text{-}205^{hi}CD8^{lo}$ cells; [8]) in the SDLN 18 h after $1,25(OH)_2D_3$ (125 ng) application suggesting that $1,25(OH)_2D_3$ can induce the migration of DC from the skin into the SDLN. Topical $1,25(OH)_2D_3$ may even promote the migration of skin DC beyond the SDLN, as $1,25(OH)_2D_3$-treated bone marrow-derived DC preferentially migrated into the Peyer's patches after adoptive transfer (either intravenously or intradermally) when administered with diptheria toxin [9].

Reduced DC numbers in the skin have previously been linked to the induction of regulatory T cells and subsequent suppressed immune responses following the treatment of skin with agents such as UV irradiation [10]. Topical $1,25(OH)_2D_3$ (400 ng/day) administered once a day for 7 days to BALB/c mice significantly reduced contact hypersensitivity (CHS) ear-swelling responses when the experimental hapten (DNFB, dinitrofluorobenzene) was applied to the same skin site as $1,25(OH)_2D_3$ [5]. Skin exposure to calcipotriol (three times daily for 3 days (1500 ng/treatment)) before transcutaneous immunization with ovalbumin (OVA) protein also reduced CHS ear-swelling responses in mice [6]. In addition, human volunteers treated topically with the vitamin D analogue calcipotriene twice-daily had significantly suppressed CHS responses when the hapten (DNCB, di-nitrochlorobenzene) was applied to the calcipotriene-treated site [11]. Similarly, Mantoux reactions were suppressed by topical administration of $1,25(OH)_2D_3$ to the skin of human volunteers [12]. In a contrasting report, $1,25(OH)_2D_3$ application after UV irradiation of Skh:hr1 hairless mice (albino), reversed the immunosuppressive effects of UV irradiation on CHS responses to oxazalone [13]. However, we have not been able to reproduce these results using a non-hairless mouse (BALB/c).

In our studies, the shaved backs of BALB/c mice were irradiated with 8 kJ/m^2 UVB and then treated immediately with $1,25(OH)_2D_3$ (125 ng). Four days later, mice were sensitised on their shaved abdomens with an experimental hapten (0.5% DNFB, see Fig. 1A). After a further 5 days, the ears of mice were challenged with 0.2% DNFB and ear swelling measured 24 h later. As expected, UVB irradiation significantly suppressed ear-swelling responses by ~40%. Topical $1,25(OH)_2D_3$ had a more modest and non-significant effect on ear-swelling responses (Fig. 1A). When topical $1,25(OH)_2D_3$ was applied immediately (or even 24 h, data not shown) after UVB irradiation, there was no reversal in the suppression caused by UVB (Fig. 1A). Furthermore, $1,25(OH)_2D_3$ had an additive effect with UVB irradiation to suppress the ear-swelling responses to a greater degree than UVB alone (Fig. 1A). Similar results were obtained using the hapten oxazalone (data not shown). In contrast and more in line with a homeostatic role for vitamin D, topical $1,25(OH)_2D_3$ after UVB irradiation consistently reversed the inflammatory effects of UVB irradiation on lymph node hypertrophy, where the number of SDLN cells/mouse was determined 4 days after the initial skin treatment (Fig. 1B). This provides an important distinction between UVB and $1,25(OH)_2D_3$. In BALB/c mice, $1,25(OH)_2D_3$ reverses the inflammatory but not the immunomodulatory effects of UV irradiation of skin.

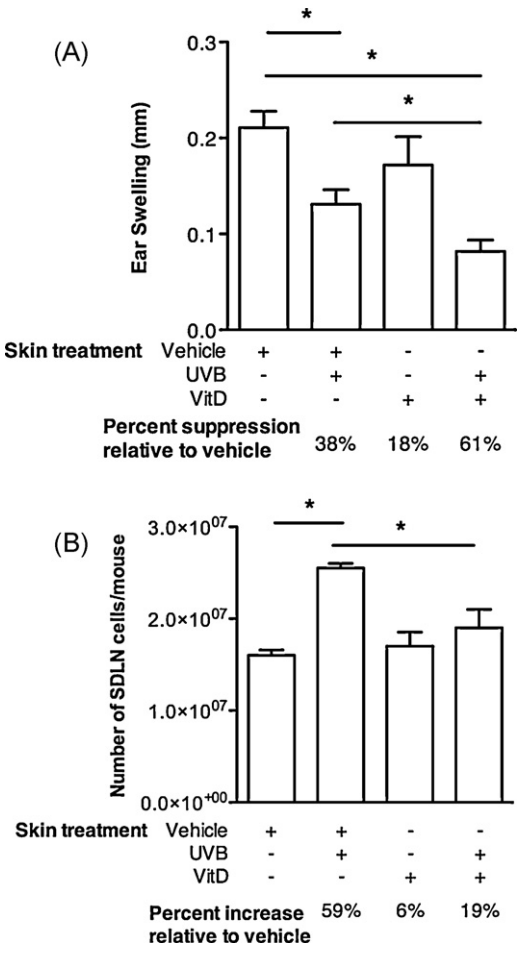

Fig. 1. Topical $1,25(OH)_2D_3$ following UVB irradiation has an additive effect on CHS responses induced by DNFB. Female 8-week-old BALB/c were shaved to remove hair from a 4×2 cm^2 area on the backs of mice. Mice were then treated with one of the following treatments; 100 μl vehicle (2:1:1, ethanol, propylene glycol, water), 8 kJ/m^2 UVB (see Ref. [14]) immediately followed by vehicle, 100 μl $1,25(OH)_2D_3$ (125 ng, VitD) or 8 kJ/m^2 UVB immediately followed by $1,25(OH)_2D_3$. In (A), 4 days after initial skin treatment, the shaved ventral skin of mice was sensitised with 25 μl 0.5% DNFB (in acetone). After a further 5 days, an ear-swelling response was initiated by administering 10 μl per ear pinnae of 0.2% DNFB (in acetone). Ear swelling was measured 24 h after ear challenge, with results for mice challenged only with 0.2% DNFB subtracted from each treatment (~0.01 mm). Results are shown as mean + SEM for 5 mice per treatment. In (B), 4 days after the initial skin treatment, the number of SDLN cells was determined after a single cell preparation was generated as previously described (see Ref. [14]) for pooled cells of three mice per treatment for three repeated experiments. Results are shown as mean + SEM. Significant differences ($p < 0.05$) are denoted by an asterisk (*) and were determined using a student's *t*-test.

A fundamental disparity in the mouse strains used may explain the differences in our findings and those previously published [13]. Like Skh:hr1 mice, vitamin D receptor (VDR) knockout mice are also hairless, perhaps indicating that expression of the VDR may be dysregulated in the skin of Skh:hr1 mice. Further, the ability of $1,25(OH)_2D_3$ to reverse UVB-induced immunosuppression in Skh:hr1 mice may occur through the non-genomic "rapid-response" pathway and not through one dependent on the genomic VDR [13]. The presence of the VDR in the skin of BALB/c mice and humans [11], where calcipotriol also suppresses CHS responses, may thus enable some of the suppressive actions of topical $1,25(OH)_2D_3$. In addition, in recent studies, $1,25(OH)_2D_3$ application immediately following UV irradiation did not reverse the immunosuppressive effects of UV irradiation on Mantoux reactions [12].

4. Topical vitamin D modifies T cell responses in the draining lymph nodes

Either through direct passage from the skin through the lymphatics, or by interacting with migrating cells (such as DC), other immune cells in the SDLN may be altered by topical $1,25(OH)_2D_3$. In particular, T cell responses are diminished following topical $1,25(OH)_2D_3$ treatment. $1,25(OH)_2D_3$ (1–2 μg) applied to hind footpad dorsal skin 5 days after foot-pad immunization with hepatitis B surface antigen modified the ability of draining lymph node (DLN) cells stimulated with anti-CD3 mAb to secrete cytokines (reduced IL-2 and IFNγ, but increased IL-4, IL-5 and IL-10) [14]. Topical $1,25(OH)_2D_3$ treatment also significantly reduced the ability of OVA-specific CD4+ T cells from OVA-TCR transgenic mice (DO11.10 mice) to proliferate and produce IL-2 when stimulated *ex vivo* [15]. In more recent studies, skin exposure to calcipotriol (3 times daily for 3 days (1500 ng/treatment)) before transcutaneous immunization with OVA protein and CpG adjuvant prevented OVA-specific CD8+ T cell priming [6]. These observations may be explained by the ability of topical $1,25(OH)_2D_3$ or calcipotriol to modify the suppressive activity or numbers of CD4+CD25+Foxp3+ cells in the SDLN [6,15].

5. Regulatory cell function is enhanced after topical vitamin D

Topical $1,25(OH)_2D_3$ enhances the suppressive capacity of CD4+CD25+(Foxp3+) cells residing in the SDLN of mice not otherwise exposed to antigen. This effect was maximal 4 days post treatment with $1,25(OH)_2D_3$ (125 ng), where CD4+CD25+ cells purified from the SDLN of $1,25(OH)_2D_3$-treated mice had an augmented ability to suppress the proliferation of co-cultured T cells, and CHS responses when adoptively transferred into naïve mice, compared to cells from vehicle-treated mice [15]. Chronic treatment of mice with calcipotriol and OVA applied to the same site resulted in the expansion of OVA-specific regulatory T cells in the DLN [6]. Chronic topical calcipotriol also increased RANKL (receptor activator of NF-κB ligand) expression by keratinocytes in a VDR-dependent manner [6]. RANKL expression has previously been linked with the expansion of regulatory T cells following UV irradiation [16].

6. Conclusion

Topical $1,25(OH)_2D_3$ and vitamin D analogues may control the pathogenesis of inflammatory skin disease by affecting the growth of keratinocytes and skin cells as well as regulating immune responses mediated by DC and T cells. By effects on DC, topical $1,25(OH)_2D_3$ can suppress CHS responses. Furthermore, $1,25(OH)_2D_3$ can reverse the inflammatory but not the immunosuppressive effects of UV irradiation of skin on immune responses. In different experimental models, $1,25(OH)_2D_3$ can also enhance the number and suppressive activity of CD4+CD25+ regulatory T cells. However, the treatment dose is an important consideration as very high doses of $1,25(OH)_2D_3$ or calcipotriol (>1500 ng/day) for 16 consecutive days to the ear skin of mice induced an atopic dermatitis-like syndrome characterized by an influx of inflammatory cells into the ears [17]. Further investigations into the mechanisms by which topical $1,25(OH)_2D_3$ acts upon immune cells in the skin and SDLN could help us better understand the pathogenesis of inflammatory skin diseases and perhaps identify new treatments in the future.

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