Effect of Topical Vitamin D Analogue on In Vivo Contact Sensitization

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Background: The immunomodulatory role of vitamin D and its analogues has been demonstrated in vitro and in vivo using animal models. We evaluated the effect of a vitamin D analogue, calcipotriene, in vivo on human subjects using a contact hypersensitivity model.

Observations: Subjects were pretreated with topical calcipotriene, simulated solar radiation, or both on buttock skin. They were then sensitized and challenged using the contact allergen dinitrochlorobenzene.

Immune response was measured by change in skinfold thickness before vs after elicitation across the challenge sites.

Conclusions: Calcipotriene-treated individuals demonstrated 64% immunosuppression compared with untreated controls. This is equivalent to the immunosuppression induced by UV exposure.

Arch Dermatol. 2006;142:1332-1334

UV irradiation causes production of vitamin D in the skin and conversion of provitamin D₃ (7-dehydrocholesterol) to the active vitamin D₃ metabolite (1,25-dihydroxyvitamin D₃). Exposure to UV radiation, even at suberythemogenic doses, also causes cutaneous immunosuppression, which has been associated with skin cancer formation. UV immunosuppression is a result of multiple biological and cellular pathways. Specific cutaneous chromophores and an array of cytokine responses are involved. Recent studies investigating the role of vitamin D and its related analogues now demonstrate that it may be responsible for modulating at least some of the immunosuppressive effects seen after UV irradiation. Cutaneous immunity largely depends on the proper functioning of epidermal Langerhans cells (LCs), which take up and process foreign antigen and migrate to regional lymph nodes for antigen presentation to T lymphocytes. Before exposure to foreign antigen, LCs are immature and have weak T-lymphocyte stimulatory power. After antigen exposure, LCs undergo a maturation process in which they develop increased levels of class II major histocompatibility complex and costimulatory ligands. Exposure to UV radiation (280-320 nm of UV-B) has been shown to alter the number, morphologic features, and antigen-presenting function of LCs and to suppress contact hypersensitivity (CHS). Vitamin D and its related analogues have been shown to play immunomodulatory roles in vitro and in vivo using animal models. Vitamin D and vitamin D receptor ligands inhibit differentiation and induce a persistent state of immaturity in dendritic cells via alterations in surface ligands and production and release of cytokines. They also induce the generation of CD4⁺ CD25⁺ T-regulatory cells, whose function is to secrete inhibitory cytokines and inhibit antigen-specific T-cell activation. All of these effects have been shown to depend on the presence of the vitamin D receptor. Cholecalciferol administration in vivo to mice markedly increased transforming growth factor β1 and interleukin 4 transcripts and caused decreased levels of interferon γ and tumor necrosis factor α gene expression. When calcipotriene was applied to human skin in vivo for 4 days, it resulted in a dose-dependent depletion in CD1a⁺ LCs with dendritic morphologic features and in the number of dendrites per cell. In the present study, we examined whether the application of topical calcipotriene would alter the CHS response in vivo in human volunteers after exposure.
to a known allergen. A well-established model for measuring CHS suppression was applied using the allergen dinitrochlorobenzene.6

METHODS

Twenty healthy volunteers with Fitzpatrick skin types I to V and no history of skin disease were recruited for the study. Participants were not taking photosensitizing or immunomodulating medications. They were randomized into 4 groups by drawing names. Fifteen participants in the 3 study groups were pretreated on buttock skin with (1) topical calcipotriene twice daily for 1 week, (2) topical calcipotriene twice daily for 1 week plus simulated solar radiation (SSR) on day 5 using 50% of the participant's minimum erythema dose (0.5 MED), or (3) 0.5 MED of SSR alone. Three days later, participants were sensitized to dinitrochlorobenzene at the treated site. The site was cleansed with alcohol and allowed to air-dry before application of the patch. The Finn chamber contained 48 µL of 0.0625% dinitrochlorobenzene and was worn for 48 hours. Two weeks later, participants were challenged with 5 increasing concentrations (from 0% to 0.0625% dinitrochlorobenzene) on the contralateral upper inner arm. The other 5 participants served as controls and received no calcipotriene ointment or SSR but underwent dinitrochlorobenzene sensitization and challenge. Using a micrometer (Mitutoyo Corp, Kawasaki, Japan), the immune response for all the groups was measured as the total millimeter increase in skinfold thickness (SFT) of the challenge sites (ie, postelicitation SFT minus preelicitation SFT).

RESULTS

The controls demonstrated a strong immune response to the allergen, with a mean increase in SFT of 4.41 mm. Individuals pretreated with calcipotriene alone or calcipotriene plus SSR showed suppression of CHS, with only a 1.6- and 1.42-mm increase in SFT, respectively (Figure 1). As expected, those who received SSR alone also had suppression, with a less than 2-mm increase in SFT (data not shown). The percentage of immunosuppression was then calculated from SFT based on the following equation:

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\% \text{Immunosuppression} = \left( \frac{\text{SFT}_{\text{control}} - \text{SFT}_{\text{treatment}}}{\text{SFT}_{\text{control}}} \right) \times 100.
\]

Our calculations demonstrated that pretreatment with calcipotriene (a vitamin D analogue) led to 64% immunosuppression relative to untreated controls. Clinically, calcipotriene-treated individuals demonstrated less erythema and edema at the skin sites challenged with dinitrochlorobenzene (Figure 2).

COMMENT

The results of this in vivo study support those of previous studies demonstrating the immunomodulatory properties of vitamin D analogues. Participants who received calcipotriene exhibited suppression of CHS when sensitized and challenged with a contact allergen. The level of immunosuppression observed was not different from that induced by a suberythemogenic UV dose. Treat-

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**Figure 1.** Mean change in skinfold thickness after dinitrochlorobenzene challenge. 0.5 MED indicates 50% of the participant’s minimum erythema dose; SSR, simulated solar radiation. Error bars represent SD.

**Figure 2.** Dinitrochlorobenzene challenge results in a control subject (A) and after pretreatment with calcipotriene (a vitamin D analogue) (B).
ing patients with calcipotriene and 0.5 MED of SSR did not result in an additive immunosuppressive effect. Mechanisms related to receptor binding and saturation should be investigated in additional studies.

DNA damage and immunosuppression are key pathways by which UV exposure leads to cutaneous malignancies. The direct contribution of UV-induced vitamin D synthesis to these processes warrants further study. This is the first in vivo human study to confirm what has been previously shown in vitro and in animal models: the vitamin D system is involved in immunomodulation. These data indicate that although vitamin D is known to have cytoprotective effects and the capacity to regulate cellular differentiation, it could also exert direct immunomodulatory effects on the skin. It may be that UV-induced vitamin D production plays a more substantial role in UV immunosuppression than was previously perceived.

Accepted for Publication: March 21, 2006.
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Author Contributions: Dr Baron had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Hanneman, Scull, Cooper, and Baron. Acquisition of data: Hanneman and Scull. Analysis and interpretation of data: Hanneman, Scull, Cooper, and Baron. Drafting of the manuscript: Hanneman, Scull, and Baron. Critical revision of the manuscript for important intellectual content: Hanneman, Scull, Cooper, and Baron. Statistical analysis: Cooper. Obtained funding: Cooper. Administrative, technical, and material support: Hanneman, Scull, Cooper, and Baron. Study supervision: Hanneman, Scull, Cooper, and Baron.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grant NIH-AR39750 from the Skin Study Center Translational Research Laboratory of the Skin Disease Research Center.

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