Vitamin D as a Therapeutic Option for Sunburn: Clinical and Biologic Implications

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Introduction

VITAMIN D₃ (CHOLECALCIFEROL) is a fat-soluble steroid hormone obtained from food sources and produced locally in the skin from the ultraviolet radiation (UVR)-dependent conversion of cholesterol precursors to the inactive form of vitamin D₃ (Bikle, 2011). In addition to its classically described functions related to the regulation of calcium homeostasis and bone metabolism, vitamin D₃ has numerous nonclassical effects, including the ability to modulate immune responses (Mora *et al.*, 2008; Wobke *et al.*, 2014). Specifically, vitamin D₃ induces monocyte to macrophage differentiation, enhances antimicrobial activity, stimulates autophagy, and suppresses the production of proinflammatory cytokines (Liu *et al.*, 2006; Fabri *et al.*, 2011; Di Rosa *et al.*, 2012; Zhang *et al.*, 2014). However, it was previously unknown whether vitamin D₃ was capable of modulating acute inflammation in humans. In a randomized, double-blinded, placebo-controlled pilot study, our group recently demonstrated that vitamin D₃ reduces skin inflammation when administered to humans 1 h after sunburn (Scott *et al.*, 2017). This commentary will attempt to outline the major clinical and biologic implications of using vitamin D as an anti-inflammatory therapeutic agent.

Clinical Implications

In our pilot study, vitamin D₃ administered one hour after sunburn reduced skin redness, as well as the levels of proinflammatory cytokines in the skin, including tumor necrosis factor (TNF)- α and inducible nitric oxide synthase (iNOS) (Scott *et al.*, 2017). Moreover, subjects with the largest rise in serum vitamin D₃ levels after intervention demonstrated consistent upregulation of genes involved in skin repair and wound healing (Scott *et al.*, 2017).

Importantly, the results from our study do not imply that photoprotective behaviors should now be substituted for high-dose vitamin D₃. The practice of strict photoprotection, including the regular use of sunscreen, remains essential in preventing the acute and chronic effects of UVR, including sunburn, photocarcinogenesis, and photoaging (Lim *et al.*, 2017; Young *et al.*, 2017). Moreover, while frequent sunscreen use does not appear to decrease serum vitamin D₃ levels or lead to vitamin D₃ deficiency, UVR-mediated

vitamin D₃ production in the skin is associated with DNA damage (Linos *et al.*, 2012; Petersen *et al.*, 2014). As such, the American Academy of Dermatology recommends obtaining adequate vitamin D₃ through supplementation rather than unprotected exposure to UVR (AAD, 2009).

In addition, vitamin D₃ was administered to subjects in our study after sunburn had already occurred (Scott *et al.*, 2017). Whether vitamin D₃ can act in an anti-inflammatory manner when given before the development of sunburn remains an unanswered question. Moreover, DNA damage would have already occurred in the skin by the time vitamin D₃ was administered. Immune surveillance is critical in eliminating DNA-damaged keratinocytes during the early stages of photocarcinogenesis, as evidenced by the increased risk for skin cancer in immunosuppressed transplant recipients, as well as the efficacy of proinflammatory immune modifiers for the treatment of premalignant skin lesions (Euvrard *et al.*, 2003; Ooi *et al.*, 2006; Cunningham *et al.*, 2017). Given that vitamin D₃ suppresses inflammation following sunburn, it is theoretically plausible that vitamin D₃ may also limit the immune-mediated clearance of DNA-damaged keratinocytes, thus allowing them to survive, proliferate, and ultimately confer a greater risk for developing skin cancer.

The optimal dose and dosing frequency for vitamin D₃ supplementation are also unclear, with specific recommendations varying by professional society (Holick *et al.*, 2011; Ross *et al.*, 2011). Randomized controlled trials have demonstrated a clinical benefit of supplementing with up to 500,000 international units (IU) of vitamin D₃ annually, and supplementation doses ranging from 400 to 4000 IU/day are generally considered safe (Dawson-Hughes *et al.*, 2010; Sanders *et al.*, 2010; van Groningen *et al.*, 2010). However, these recommendations are extrapolated from the benefits of vitamin D₃ supplementation on bone health, and the ideal dosing strategy required to achieve immunomodulatory effects *in vivo* is still unknown (Sohl *et al.*, 2015). We administered a one-time, postexposure vitamin D₃ dose ranging from 50,000 to 200,000 IU and observed no adverse events during the duration of the study period (Scott *et al.*, 2017). Serum calcium and vitamin D₃ levels remained within the recommended reference range for all subjects throughout the duration of the study, supporting that single high doses of vitamin D₃ are both safe and well tolerated as therapeutic interventions.

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Finally, the pharmacokinetics of a single, large dose of vitamin D₃ is complex. An individual's response to a large bolus of vitamin D₃ will depend on several factors, including body mass index (BMI), genetic polymorphisms, and baseline vitamin D₃ stores, among others (Ilahi *et al.*, 2008; Didriksen *et al.*, 2013). Our study was not statistically powdered to match subjects by their baseline vitamin D₃ levels, and subjects' baseline serum vitamin D₃ levels ranged widely from 18.4 to 36.5 ng/mL (Scott *et al.*, 2017). As a result, it remains unclear whether individuals with lower baseline stores require higher vitamin D₃ doses to achieve therapeutic effects. Similarly, as BMI also varied widely from 21.3 to 41.6, it also remains unclear whether individuals with higher BMI require higher vitamin D₃ doses due to the potential for distribution in adipose tissue (Scott *et al.*, 2017). Therefore, the results of our pilot clinical trial will need to be replicated in larger, diverse populations of subjects before vitamin D₃ can be recommended for its anti-inflammatory properties in routine clinical practice.

Biologic Implications

At the tissue level, sunburn results in vasodilation and dermal edema, which manifest clinically as erythema and swelling, respectively. At the cellular and molecular level, sunburn is characterized by an influx of neutrophils and macrophages into the skin, as well as the release of pro-

inflammatory mediators, including TNF- α (Fig. 1) (Gilchrest *et al.*, 1983; Cooper *et al.*, 1993; Clydesdale *et al.*, 2001). Moreover, when exposed to inflammatory mediators, skin-infiltrating macrophages differentiate into proinflammatory, M1-polarized macrophages (Sethi and Sodhi, 2004; Mills, 2012). M1-macrophages produce iNOS as part of an antimicrobial response during periods of skin inflammation (Mills, 2012). However, excessive production of iNOS increases tissue damage by prolonging inflammation and preventing tissue repair (Mills, 2012). In contrast to proinflammatory M1-macrophages, M2-polarized macrophages are anti-inflammatory and function to promote tissue repair and wound healing (Mills, 2012).

Other groups have demonstrated *in vitro* that monocytes treated with vitamin D₃ and retinoic acid differentiate into M2-macrophages (Di Rosa *et al.*, 2012; Takahashi *et al.*, 2014). Keratinocytes and macrophages both possess 25-hydroxyvitamin D₃ 1-alpha-hydroxylase (CYP27B1), an enzyme that allows these cells to convert vitamin D₃ into its active form (Adams and Gacad, 1985). This conversion occurs extrarenally, which unlike the proximal tubule of the kidney, is not under feedback inhibition (Adams and Gacad, 1985; Zhang *et al.*, 2012). A mechanism for locally producing active vitamin D₃ suggests that vitamin D₃ may signal in an autocrine or paracrine manner to modulate immune responses within the skin microenvironment. Moreover, endogenous retinoids, commonly found in the skin, are increased in the skin of mice following UVR exposure, and have also been

FIG. 1. Schematic of vitamin D₃ role in mediating anti-inflammation and tissue repair following ultraviolet injury of the skin.

shown to be required for skin repair following acute UVR exposure (Everts, 2012; Gressel *et al.*, 2015).

We propose that administering a large bolus of vitamin D₃ after sunburn increases the local concentration of inactive vitamin D₃ within sunburned skin, which is then rapidly converted to active vitamin D₃ by keratinocytes and skin-infiltrating macrophages (Fig. 1). Active vitamin D₃, combined with endogenous retinoids generated from exposure to acute UVR, results in the preferential differentiation of anti-inflammatory M2-macrophages. The presence of M2-polarized macrophages within sunburned skin helps resolve inflammation, promote tissue repair, and restore homeostasis. In our study, M2-macrophages expressing arginase-1 and markers of autophagy were increased in sunburned skin following vitamin D₃ intervention (Scott *et al.*, 2017).

Furthermore, pharmacological inhibition of autophagy increases apoptosis following acute UVR exposure, suppresses the recruitment of M2-macrophages into sunburned skin, and prevents the vitamin D₃-dependent downregulation of proinflammatory mediators (article under review). Taken together, these data support a critical role for M2-macrophages with enhanced autophagy in mediating the anti-inflammatory and protective effects of vitamin D₃ after sunburn. Interestingly, a similar protective role for M2-macrophages after treatment with vitamin D₃ has been proposed in a rat model of diabetic nephropathy (Zhang *et al.*, 2014). However, to validate this hypothesis, it will be important to determine if a rise in the serum vitamin D₃ concentration following high-dose vitamin D₃ intervention is associated with a concomitant rise in the local vitamin D₃ concentration within the skin.

Multiple adaptations exist within mammalian skin to protect against the harmful effects of UVR that impair the barrier function of skin. These include a stratified squamous epithelium comprising keratinocytes linked together by desmosomes, multiple layers of anucleated corneocytes bound together tightly in the stratum corneum, melanin pigmentation, and immune surveillance in the form of antigen-presenting cells within the epidermis and dermis that recognize and respond to danger- and pathogen-associated molecular patterns (Clydesdale *et al.*, 2001). Interestingly, there is an evolutionarily conserved mechanism for both generating inactive vitamin D₃ within the skin, as well as a mechanism for rapidly activating vitamin D₃ by skin-resident cells independent of systemic circulation and renal metabolism (Bikle, 2011). It is plausible that these mechanisms arose and persisted throughout our evolutionary history because of a critical role for vitamin D₃ in maintaining skin homeostasis. By virtue of its exposed location, skin is constantly exposed to inflammatory environmental insults, and vitamin D₃ may therefore function as a complementary defense mechanism to suppress low levels of skin inflammation and restore skin barrier function.

In summary, the results of our *in vivo* human study, combined with animal and *in vitro* models, suggest that vitamin D₃ exerts immunomodulatory effects at the cellular and tissue level by selectively inducing the differentiation of anti-inflammatory, M2-macrophages. M2-macrophages, through the expression of arginase-1, downregulation of TNF- α and iNOS, and induction of autophagy, boost the resolution of inflammation, promote tissue repair, and enhance wound healing. Thus, vitamin D₃ may serve a unique role in the skin,

acting as an “endocrine barrier” to provide additional protection against environmental injury and effectively maintain skin barrier function.

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