Original article:
UVB Dose Optimization for Phototherapy in Vitamin D Deficiency: Profile Analysis of Vitamin D, TNF-α, Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor (PDGF) in Wistar Rats

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Abstract:
Background: UVB radiation responsible for the most important biological effects including Vitamin D3 synthesis and inflammation. UVB radiation are absorbed by 7-dehydrocholesterol in the plasma membrane of epidermal cells resulting in production of cis-previtamin D3. In the other hand, an exposure to UVB leads to cutaneous tissue inflammation modulates by TNF-α which also increases platelet activating factor. VEGF and PDGF induced by TNF-α during wound healing, characterized with angiogenesis and reepithelialization. Furthermore, vitamin D plays a role in inflammation inhibition and upregulates growth factors. However, the study of the mechanism has not yet been thoroughly investigated. Methods: This study uses post test only group design, subjected wistar rats divided into four groups. Control group, non irradiated with UVB, and the other three groups, treated with graded UVB dose started with 1 MED (50 mJ/cm²), 2 MED (100 mJ/cm²) and 3 MED (150 mJ/cm²) and investigated at 6, 12, 24 and 48 hours post UVB irradiation. Result: The serum level of vitamin D, VEGF and PDGF were increasing due to UVB dose addition. The highest level was reached at 6 hours post radiation using 3 MED, which gradually decrease up to 48 hours (p =0.000). The rise of vitamin D after UVB radiation, inhibit TNF-α induction in every dose accordant UVB dose addition and the lowest level is using 3 MED at 12 hours post radiation (p =0.000). TNF-α reach its highest level at 24 hours post radiation using 1 MED, it is related with the acute phase of inflammation. Conclusion: This study reveal that higher UVB irradiance increases vitamin D and inhibit TNF-α which also promotes VEGF and PDGF.

Keywords: UVB, Vitamin D, TNF-α, VEGF, PDGF

Introduction

Being a component of the electromagnetic spectrum, UV photons wavelengths between visible light and gamma radiation. UV energy subdivided into UV-A, -B and -C with UV-C photons having the shortest wavelengths (100–280 nm) and highest energy, UV-A having the longest (315–400 nm) but least energetic photons and UV-B falling in between. Each component of UV can exert a variety of effects on cells, tissues and molecules. UVB is almost completely absorbed by the epidermis, with comparatively little reaching the dermis. UVB is directly absorbed by DNA which causes molecular rearrangements forming the specific photoproducts such as cyclobutane dimers and 6–4 photoproducts. Mutations and cancer can result from many of these modifications to DNA¹.

UVB radiation, comprising approximately 5–10% of the entire spectrum of UV radiation reaching the surface of the earth and responsible for the most

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important biological effects including vitamin D3 synthesis, inflammation, and carcinogenesis. UVB is absorbed in the stratum corneum of the skin by cellular chromophores. Effected factors in the skin are melanin, cellular DNA, urocanic acid, proteins, lipids, and amino acids. During sun exposure UVB radiation with wavelengths of 290-315 nm are absorbed by 7-dehydrocholesterol in the plasma membrane of epidermal cells resulting in production of cis-previtamin D3. This thermodynamically unstable molecule begins to rapidly isomerize by a non-enzymatic membrane-enhanced process within a few hours to vitamin D3. Once formed, vitamin D3 exits the circulation and is transported to the liver where it is converted to 25-hydroxyvitamin D3 [25(OH)D].

UVB induced inflammatory response is mainly mediated by tumor necrosis factor-α (TNF-α). TNF-α also increases lipid signal transduction mediators such as prostaglandins and platelet activating factor. Based on these roles, TNFα has been proposed as a central player in inflammatory cell activation and suggested to play a critical role in the development of many chronic inflammatory diseases. In the skin, TNF-α modulates the initial stages of the response to inflammation and injury. The stimulation of keratinocytes by TNF-α leads to the activation of various signaling pathways that involve caspases, nuclear factor-kappa B (NF-κB), and mitogen activated protein kinases (MAPKs), which subsequently increase the expression of inflammatory mediators. The healing of soft tissues after inflammation involves reepithelialization and angiogenesis. Epidermal keratinocyte expresses and release immunomodulatory mediator as a response from UVB radiation, allergen, hapten, microbiologic agents, and cytokine such as tumor necrosis factor (TNF)-α dan interferon (IFN)-γ.

VEGF are induce by TNF-α and strongly expressed by epidermal keratinocyte during wound healing, characterized with microvascular permeability increases and angiogenesis. Another growth factor such as PDGF also plays a role in wound healing as a response from inflammation. PDGF stimulates the production and secretion of collagenase by fibroblasts, suggesting a role in the remodeling phase of wound healing. Early observations revealed that PDGF is released by platelets and secreted by activated macrophages, smooth muscle cells of damaged arteries, as well as by epidermal keratinocytes.

Vitamin D homeostasis seems to be critical for many tissues to maintain normal proliferation and differentiation. Cellular studies shown that vitamin D is a key modulator of immune function and inflammation. There is an appreciation that vitamin D exerts broad regulatory effects on cells of the adaptive and innate immune system. Current evidence found that the circulating level of 25(OH)D may be crucial for the optimal anti-inflammatory response of human monocytes. The conversion of 25(OH)D to its active form 1,25(OH)2D occurs locally in immune system cells. The active metabolite of vitamin D has an anti-inflammatory effect on the inflammatory profile of monocytes, down-regulating the expression and production of several pro-inflammatory cytokines including TNF-α.

Furthermore, vitamin D inhibits the activation of TNF alpha converting enzyme (TACE). TACE activation in renal cells gives rise to subsequent release of TNF-alpha, into the circulation, promoting systemic inflammation. However, activation of TACE can be blocked by active vitamin D preparations. TACE activation is usually secondary to activation of the renal RAS system, which is also directly inhibited by VDR activation. Thus, vitamin D suppresses TACE activation and subsequent inflammation on multiple levels.

Materials and Methods

Subjected Rats

10-12 weeks male wistar rats weighed from 160-180 gr. Provided by Inter-University Center Building of Gajah Mada University Yogyakarta. All 24 rats was divided into 4 groups. Control group, was non irradiated with UVB, and the other three groups were irradiated with graded UVB dose starting at 1 MED (50 mJ/cm2), 2 MED (100 mL/cm2) and 3 MED (150 mL/cm2). The dorsal skin were shaved and irradiated with UVB accordant to the dose. Serum sample was collected at 6, 12, 24, and 48 hours post radiation, using ELISA to analyze the vitamin D, TNF-α, VEGF and PDGF profiles.

UVB Lamp

Phillips narrowband UVB type PL-S 9W/01/2P. The height of the lamps was adjusted to deliver 50, 100 and 150 mJ/cm2.
Statistical Analysis

To determine the difference between variables significantly we use one way anova study in each variables at every time series.

Results

a. Effects of UVB Radiation Dosage to Vitamin D

![Vitamin D Level at 6, 12, 24 and 48 hours post sham-irradiated](image1)

After sham-irradiated, vitamin D profile began to rise start with 1 MED, and increased at 6 hours post radiation (16.19±0.4) which gradually decrease after 48 hours (13.25±0.37). Followed with 2 MED which also increased at 6 hours post radiation (16.98±0.5) and gradually decrease at 48 hours (14.78±0.42). The highest vitamin D level achieved using 3 MED at 6 hours post radiation (17.98±0.34) and gradually decrease to the next 48 hours (17.06±0.37).

b. Effects of UVB Radiation Dosage to TNF-α

![TNF-α Level at 6, 12, 24 and 48 hours post sham-irradiated](image2)

TNF-α profile decreasing after radiation start with 1 MED at 6 hours post sham-irradiated(41.63±2.08), then slightly increase in the next 12 hours (42.85±0.71) and reached it peak at 24 hours post sham-irradiated (47.13±1.12) during acute phase of inflammation, and decreases for the next 48 hours (43.39±1.12).

c. Effects of UVB Radiation Dosage to VEGF

![VEGF Level at 6, 12, 24 and 48 hours post sham-irradiated](image3)

VEGF profile increases accordance with UVB dose addition started with 1 MED at 6 hours post radiation (24.37±0.8) and decreases after 48 hours (20.26±0.96). Followed by 2 MED at 6 hours post radiation (25.46±0.8) and decreases for the next 48 hours (22.21±0.92). The highest VEGF profile achieved using 3 MED at 6 hours post radiation (27.59±0.46) and decreases after 48 hours (26.78±0.47).

d. Effects of UVB Radiation Dosage to PDGF

![PDGF Level at 6, 12, 24 and 48 hours post sham-irradiated](image4)

PDGF profile increases accordance with UVB dose addition started using 1 MED at 6 hours post sham-irradiated (37.47±0.65) and decreases after 48 hours (36.71±0.44). Followed by 2 MED at 6 hours post radiation (41.53±1.25) and the highest profile was using 3 MED at 6 hours post radiation (44.85±0.72).
Discussion

Effects of UVB Radiation Dosage to Vitamin D

UVB radiation, responsible for the most important biological effects including vitamin D3 synthesis and inflammation.\textsuperscript{2} UVB radiation are absorbed by 7-dehydrocholesterol in the plasma membrane of epidermal cells resulting cis-previtamin D3.\textsuperscript{3,4} This unstable molecule begins to isomerize by a non-enzymatic membrane-enhanced process within a few hours to vitamin D3.\textsuperscript{5,6}

Holick and co-workers found that one full body UV exposure causing a slight pinkness in skin (one minimum erythemal dose, 1 MED) is equivalent to an oral intake of somewhere in the range 250–625 μg (10,000–25,000 IU) of vitamin D3.\textsuperscript{31-33} Chel et al, obtained very similar results for a half MED exposure of 1000cm2 (one-sixth of the body area) yielding the equivalent of 400 IU corresponding to 9,600 IU for a full body MED exposure.\textsuperscript{34}

In this study we found that, serum level of vitamin D were increased due to UVB dose addition begin with 1 MED, followed by 2 MED, and the highest level are using 3 MED and its investigated at 6 hours post radiation, which gradually decrease up to 48 hours post radiation. It has been often claimed that adequate vitamin D is obtained after a few minutes of sun exposure. This is commonly true only when the sun is high and if the equivalent of 400 IU (corresponding to one spoonful of cod liver oil) is desired. These amounts are usually not available from casual daily exposure (e.g., waiting for the bus to work).

The gap between beneficial UV exposure to obtain desirable vitamin D and harmful exposure leading to erythema also narrows (i.e., desirable vitamin D effective dose approaches 1 MED). For example, for normal summer clothing, the exposed skin (25.5%) needs to be sunburnt in order to produce 4,000 IU. The skin would then receive a UV dose associated with clearly elevated sunburn cells and skin cancer risk.\textsuperscript{35}

Effects of UVB Radiation Dosage to TNF-α

UVB induced inflammatory response is mainly mediated by Tumor necrosis factor-α (TNF-α).\textsuperscript{7} TNFα also increases lipid signal transduction mediators such as prostaglandins and platelet activating factor.\textsuperscript{8,9} In the skin, TNF-α also modulates the initial stages of the response to inflammation and injury.\textsuperscript{10-13} According to Lee, et al, 2015, protein expression of IL-1β, IL-6 (*p<0.05), IL-8, and TNF-α in cultured sebocytes treated with 40 mJ/cm2 or 70 mJ/cm2 UVB showed more decreasing tendency after the addition of 10–6 M vitamin D compared with 10–8 M vitamin D.\textsuperscript{14} We found that during UVB exposure, started with 1 MED to 3 MED, the TNF-α serum were decreases accordant with UVB dose addition while the vitamin D serum level arising. The lowest TNF-α serum level are showed at 12 hours post radiation using 3 MED. Meanwhile, the highest performance were showed at 24 hours post radiation using 1 MED. It is related with acute phase of inflammation. Faurischou, 2010, confirm that apoptosis is not significantly increased in HaCaT cells after exposure to 10 mJ/cm2 at 4, 24, 48 hours after irradiation but its increased 24 and 48 hours after exposure to 20 mJ/cm2.\textsuperscript{35}

Effects of UVB Radiation to VEGF and PDGF

VEGF is also overexpressed by epidermal keratinocytes in certain non-neoplastic processes of the skin which are characterized by increased microvascular permeability and angiogenesis, e.g. cutaneous wound healing.\textsuperscript{36} Brauchle, et al, 1996, using HaCaT cells shown that within 5 h after UVB irradiation (10 and 20 mJ/cm2), a large induction of VEGF mRNA expression was observed with highest levels 8 h after exposure to 20 mJ of UVB/cm2. At this time point, VEGF mRNA levels were 11-fold higher compared with the basal level. This effect was long lasting, and 24 h after exposure of the cells to UVB, VEGF mRNA levels were still elevated (2–3-fold).\textsuperscript{37}

Prior reports suggest that following UV irradiation VEGF in keratinocytes is induced primarily by tumor necrosis factor (TNF)-α, a cytokine synthesized and secreted by keratinocytes after UV irradiation. Several growth factors and cytokines induce VEGF expression in epidermal keratinocytes. These include platelet-derived growth factor, tumor necrosis factor α (TNF)-α, and keratinocyte growth factor.\textsuperscript{38}

Using SCC-12F cells, Kosmadaki et al., 2003, found that VEGF mRNA in sham-irradiated keratinocytes increase gradually in band intensity with culture maturation within 16 h of UV. Peak upregulation was observed as early as 24 h after exposure and persisted through 48 h, when the experiment was terminated were irradiated with 30 mJ/cm2 of UVB.\textsuperscript{39} Compare with our study, VEGF were increased gradually accordant with UVB dose addition and reach the highest at 6 hours after radiation using 3 MED and decrease up to 48 hours post radiation. The same result shown in PDGF.
Conclusion

In summary, UVB radiation responsible to several events include vitamin D synthesis and inflammation. The higher UVB irradiance increases vitamin D and plays its role as a key modulator in inflammation and inhibit TNF-α which also promotes VEGF and PDGF in wound healing process after tissue injury caused by UVB irradiation.

Ethical Clearance

All procedures performed involving animal were approved by Komisi Etik Penelitian Kedokterandan Kesehatan (Medical and Health Research Ethics Committee) Faculty Medicine of Universitas Islam Sultan Agung.

Acknowledgements

We thank to Kementerian Riset Teknologidan Pendidikan Tinggi Indonesia for Thesis Magister 2019 Research Grant.

Conflict of interest

The investigators declare that they have no conflict of interests.

Author’s contribution

Data gathering and idea owner of this study: Cynthia Arsita
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