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The Mechanisms of Action of Phototherapy in the Treatment of the most Common Dermatoses

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A B S T R A C T

Phototherapy denotes the use of ultraviolet (UV) light in the management of several dermatoses. Most phototherapy regimens utilize ultraviolet radiation of different wavelenghts. Currently, irradiations with broadband UVB (290–320 nm), narrowband UVB (311–313 nm), 308 nm excimer laser, UVA 1 (340–400 nm), UVA with psoralen (PUVA), and extracorporeal photochemotherapy (photopheresis) are being used. The interplay of the various photobiologic pathways is far from being completely understood. Disordes that may benefit from such approach are numerous, with psoriasis, atopic dermatitis, cutaneous T-cell lymphomas, morphea, and vitiligo as main indications. The immunomodulatory effects of UVB radiation primarily affect the epidermis and superficial dermis, while UVA radiation affects mid and deep dermal components, especially blood vessels. UVB radiation is absorbed by endogenous chromophores, such as nuclear DNA, which initiates a cascade of events. Absorption of UV light by nucleotides causes the formation of DNA photoproducts and supresses DNA synthesis. In addition UV light stimulates synthesis of prostaglandins and cytokines that play important roles in immune suppression. It may reduce the number of Langerhans cells, cutaneous T lymphocytes and mast cells in the dermis. UV radiation can also affect extranuclear molecular targets located in the cytoplasm and cell membrane. Immune suppression, alteration in cytokine expression, and cell cycle arrest may all contribute to the suppression of disease activity. PUVA is a form of chemophototherapy which uses UVA light to activate chemicals known as psoralens, hence psoralen ultraviolet A. The conjunction of psoralens with epidermal DNA inhibits DNA replication and causes cell cycle arrest. Psoralen photosensitization also causes an alteration in the expression of cytokines and cytokine receptors. Psoralens interact with RNA, proteins and other cellular components and indirectly modify proteins and lipids via singlet oxygen-mediated reactions or by generating of free radicals. Infiltrating lymphocytes are strongly suppressed by PUVA, with variable effects on different T-cell subsets. Psoralens and UV radiation also stimulate melanogenesis. Extracorporeal photopheresis is technique used in treatment of erythrodermic cutaneous lymphomas. It is very potent in induction of lymphocyte apoptosis. Despite the introduction of numerous effective systemic medications and biologic agents in dermatology, phototherapy remains a reliable, and often preferred option for several dermatoses.

Key words: *nonionizing light, phototherapy, photochemotherapy, ultraviolet therapy, apoptosis, photobiology, tumor suppressor protein p53, urocanic acid, NF-kappa B*

Introduction

Phototherapy denotes the use of ultraviolet (UV) radiation in the management of several dermatoses. Most phototherapy regimens utilize ultraviolet radiation of different wavelengths. The fluorescent lamps with specifically coated tubes allow filtering of different wavelengths. Initial dosimetry tests are used to determine the minimal erythema dose, and than subsequent gradually increasing doses are applied. Proper dosimetry is required to avoid acute side effects such as sunburn reaction.

Disordes that may benefit from such approach are numerous, with psoriasis, atopic dermatitis, cutaneous T-cell lymphomas, morphea, and vitiligo as main indications.

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mer laser, UVA 1 (340–400 nm), UVA plus psoralen (PUVA), and extracorporeal photochemotherapy (photopheresis) are being used[1].

UV radiation shows a spectrum of effects on human skin. The interplay of the various photobiologic pathways is far from being completely understood.

Ultraviolet B therapy

UVB is the simplest form of phototherapy, as it refers to the use of artificial UVB radiation without additional exogenous photosensitizers. The immunomodulatory effects of UVB radiation primarily affect the epidermis and superficial dermis[2]. The radiation is absorbed by the major endogenous chromophores with immunological importance, such as nuclear DNA, trans-urocanic acid, and cell membranes[1]. Absorption of UVB by nucleotides leads to the formation of DNA photoproducts, primarily pyrimidine dimers. Cyclobutane dimers constitute a majority of the total UV radiation-induced DNA damage. UVB exposure reduces DNA synthesis and is therefore used to suppress the accelerated DNA synthesis, such as in epidermal cells of patients with psoriasis. In addition to DNA effect, UVB exposure upregulates expression of tumor suppressor gene p53 resulting in either cell cycle arrest (allowing time for DNA repair) or apoptosis of keratinocytes (»sunburn cells«) in case of irreparable DNA damage[3,4]. The upregulation of this tumor suppressor gene may be responsible for inhibition of keratinocyte turnover in psoriatic plaques.

UVB light causes photoisomerization of urocanic acid (UCA), the second most important chromophore in the epidermis. UVB causes isomerization of UVC from trans-UCA to cis-UCA[5]. The presence of cis-UCA has immunosuppressive effects via cutaneous cytokine production skewing from Th-1 to a Th-2 response[6].

In addition to DNA, UV radiation can affect extranuclear molecular targets located in the cytoplasm and cell membane. These targets include cell surface receptors, kinases, phosphatases, and transcription factors. Devary et al. have found that UV response does not require a nuclear signal and is likely to be initiated at the plasma membrane. Activation of nuclear factor-kappa B at the cell membrane following UVB exposure leads to T-lymphocytes apoptosis, which requires de novo protein synthesis, and increased membrane permeability which may also play a role in immunosuppression[7].

In addition to its effect on the cell cycle, UV light induces the release of prostaglandins and cytokines. Following UVB exposure, keratinocytes synthesize important pro-inflammatory mediators such as interleukin (IL)-1 and TNF-α, which suppress Langerhans cells and thereby induce immunosuppression[8]. Several cytokines with important role in immune suppression, such as IL-6, IL-8, IL-10, IL-12, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF) and prostaglandins, are released from UV-irradiated keratinocytes. Prostaglandin E_2 (PGE_2) is a potent mediator that inhibits the expression of co-stimulatory molecules on the surface of antigen-presenting cells and thereby prevents the activation of T lymphocytes. Langerhans cells, the most important epidermal antigen-presenting cells, are highly susceptible to UVB irradiation, which reduces their number and alters their antigen-presenting function. This mechanism has been proven to be effective in treatment of atopic dermatitis and cell-mediated (type IV) contact dermatitis. UVB exerts its action through direct phototoxic effect on T-lymphocytes in the dermoepidermal junction in patients with atopic dermatitis[1]. Interestingly, UVB is also effective in suppression of Staphylococcus aureus colonization in atopic dermatitis[9]. UVB inhibits Th-1 axis by IL-12, interferon-γ (IFN-γ) and IL-8, and can selectively reduce proinflammatory cytokine production in individual T cells, exerting beneficial effect in psoriasis that is characterized by intraepidermal and perivascular T-cell infiltrates in the papillary dermis[10]. Narrowband UVB also causes decreased expression of the intercellular adhesion molecule-1 (ICAM-1), molecule significant for several inflammatory dermatoses[11].

Broadband UVB implies 280–315 nm radiation, while the lower wavelengths contribute significantly to burning. In the past two decades, use of different fluorescent tube coatings has allowed development of narrowband UVB with a narrow emission peak at 311 nm wavelength.

According to obtained results from several controlled studies, narrowband UVB phototherapy is superior to conventional broadband UVB therapy in the management of psoriasis, moderately severe atopic dermatitis and widespread vitiligo, with respect to therapeutic onset and remission duration[12,13].

Keratinocytes, circulating and cutaneous T lymphocytes, monocytes, Langerhans cell, mast cells and fibroblasts are targeted by narrowband UVB.

Ozawa et al. have demonstrated that narrowband UVB light causes greater depletion of T cells in psoriatic plaque than broadband UVB, and induces more rapid apoptosis of dermal T cells induced by narrowband UVB[2]. Low doses of UVB light suppress mast cell degranulation, histamine release and prevent the UV-induced vasodilatation, being an important therapeutical mechanism in atopic dermatitis and mastocytosis[14]. Immune suppression, alteration of cytokine expression, and cell cycle arrest may all contribute to the suppression of disease activity in psoriatic plaques.

Excimer laser emits monochromatic light at 308 nm wavelength and has a spot size of 3,2 cm^2. Several studies have shown that fewer treatments with the excimer laser in patients with localized psoriatic plaques are required for plaque regression when compared to narrowband UVB phototherapy[15]. The use of this laser is limited to treatment of localized plaques due to the small spot size, which makes targeting of large surface areas impractical[16,17].

Westerhof and Nieuweboer-Krobotova reported for the first time that twice-weekly narrowband UVB for the maximum period of one year causes follicular repigmentation in areas affected by vitiligo[18]. UV radiation en-

hances transcription of the tyrosinase gene (via microphthalmia-associated transcription factor), upregulates expression of proopiomelanocortin and its derivative peptides within keratinocytes, melanocytes and other cutaneous cells. In addition, melanocytes dendricity and melanosome transport to keratinocytes are stimulated via increased activity of Rac1 (involved in dendrite formation), increased kinesin to dynein ratio, and upregulated expression of protease-activated receptor-2 (PAR2; involved in melanosome transfer). UV radiation results in augmented anterograde transport by increased kinesin and decreased dynein activity[19,20].

The proposed mechanisms for UVB effect on cutaneous T-cell lymphomas include impairment of epidermal Langerhans cell function and alterations in cytokine production and adhesion molecule expression by keratinocytes. Narrowband UVB induces local and systemic immunosuppressive effects which may particularly contribute particularly to beneficial effect of this light source.

Ultraviolet A therapy and photochemotherapy

The UVA spectrum (320–400 nm) has been arbitrarily subdivided into two parts: UVA1 (340–400 nm) and UVA2 (320–340 nm). Because of its longer mean wavelength, UVA1 radiation penetrates more deeply into the skin than UVA2, and thus affects not only epidermal structures, but also mid and deep dermal components, especially blood vessels. UVA effects are dominated by indirect DNA damage caused by reactive oxygen species such as singlet oxygen. Apoptosis can be induced by DNA damage, withdrawal of growth cytokines (e.g. EGF, TGF-α, IGF, PDGF) and by cell-death mediators (e.g. TNF)[21]. DNA damage due to UV radiation upregulates expression of the p53 tumor suppressor gene in the basal and suprabasal layers of epidermis[22]. The p53 acts as a »guardian of the genome« being involved in several cell cycle checkpoints (G_1, G_2 and M) and this can lead to either cell cycle arrest (allowing time for DNA repair) or apoptosis of keratinocytes (»sunburn cells«) in case of irreparable DNA damage[23].

The regulation of G_1, G_2 and M transitions involes three major protein families: cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs). G_1 arrest is mainly caused by p53-mediated activation of CKI which inhibits phosphorylation of retinoblastoma (Rb) protein. Dephosphorylated Rb protein blocks keratinocyte proliferation by binding to, and thereby inactivation of transcription factor (E2F) required for transcription of G_1/S cyclins and cell cycle propagation. Rb dephosphorylation, at the G_1 checkpoint blocks cell proliferation and causes G_1 arrest[24].

The p53 protein activates transcription of pro-apoptotic proteins (BAX) and inhibits transcription of anti-apoptotic proteins (Bcl-2). BAX activates apoptosis-related proteases, termed caspases, that cause fragmentation of genomic DNA by endonucleases, and degradation of structurally important proteins such as laminin and actin[21].

Edström et al. noted an increase of Ki67 positive cells in the epidermis and a slight increase of cyclin A positive cells after each cycle of UVA1 irradiation. This positivity indicates that there is an increased proportion of epidermal cells in the G_1 phase which results in significant thickening of stratum corneum[25].

The mechanisms of apoptosis induced by PUVA and UVA-1 differ. UVA1 irradiation induces both early apoptosis (protein synthesis independent) and late apoptosis of T-lymphocytes that are relevant in the treatment of atopic dermatitis and mycosis fungoides[26]. It may reduce the number of Langerhans cells and mast cells in the dermis in atopic dermatitis, and in cutaneous mastocytosis. In addition, it has been shown that increased matrix metalloproteinase (MMP)-1 expression in treated lesions of localized scleroderma induces softening and disappearance of sclerotic skin[25].

PUVA is form of chemophototherapy which uses UVA (315–400 nm) to activate chemicals known as psoralens, hence psoralen ultraviolet A (PUVA). PUVA is used to treat numerous dermatoses among which psoriasis, aopic dermatitis, vitiligo and cutaneous T-cell lymphoma are well-established indications[27]. Oral administration of psoralen with UVA was used for many years, with increasing use of various topical regimens, such as bath and cream PUVA. The latter avoid some of the systemic effects of oral psoralen, notably nausea and corneal uptake (protective glasses are required for 24 h after oral PUVA therapy)[28,29]. Psoralens react with DNA on three levels. First, in the absence of UV radiation, the psoralen intercalates between DNA base pairs. Absorption of photons in the UVA range results in the formation of unstable complexes 3,4- or 4´,5´-cyclobutane monoadduct with pyrimidine bases of native DNA. The 4´,5´ monoadducts can absorb a second photon and this reaction leads to the formation of an interstrand cross-links in the double helix[4].

Excited psoralens can also react with molecular oxygen. The reactive oxygen species formed by this reaction cause cell membrane and mitochondria damage by lipid peroxidation and may activate the cyclooxygenase and arachidonic acid metabolic pathways.

The conjunction of psoralens with epidermal DNA inhibits DNA replication in T-lymphocytes and keratinocytes and causes cell cycle arrest which leads to subsequent inhibition of cell proliferation in several dermatoses. However, since PUVA is also effective in nonproliferative diseases, other mechanisms of action may be involved. Indeed, psoralen photosensitization also alters expression of cytokines and cytokine receptors, such as impairment of IL-2 production by T-lymphocytes, inhibition of epidermal growh factor receptor tyrosine kinase activity and inhibition of chemotactic activity of polymorphonuclear neutrophils in response to anaphylatoxin C5a[30,31].

Psoralens also interact with RNA, proteins and other cellular components and indirectly modify proteins and lipids via singlet oxygen-mediated reactions or by generating free radical production.

PUVA can reverse the pathologically altered patterns of keratinocyte differentiation markers and reduce the number of proliferating epidermal cells. Infiltrating lymphocytes are strongly suppressed by PUVA, with variable effects on different T-call subsets. PUVA is far more potent in induction of apoptosis in T lymphocytes and antigen presenting cells than in keratinocytes, which may explain its efficacy in cutaneous lymphomas as well as in inflammatory skin diseases such as psoriasis and atopic dermatitis[32]. Marks and Fox have suggested that PUVA could lead to apoptosis and modify expression of new oligopeptides in surface MHC molecules. This mechanism may be responsible for a higher level of antigenicity of these cells[33].

In addition, PUVA also mediates the downregulation in expression of »homing receptors« (HECA) of epidermotropic malignant T cell, thus PUVA serves as effective treatment modality in early-stage cutaneous T-cell lymphoma[34].

PUVA also stimulates melanogenesis. The process involves the photoconjugation of psoralens to DNA in melanocytes, followed by mitosis and subsequent proliferation of melanocytes which leads to repopulation of the epidermis, increased formation and melanization of melanosomes, enhanced transfer of melanosomes to keratinocytes, and increased synthesis of tyrosinase via stimulation of cAMP activity[18].

Extracorporeal Photopheresis

Extracorporeal photopheresis (ECP) is a technique which uses UVA irradiation of leukocyte-enriched blood in the presence of psoralen. Psoralens can be administered orally or directly to the leukocyte/plasma concentrate, which is then irradiated outside the body in the process of plasmapheresis, and returned to the circulation. It is an effective therapeutic regimen for erythrodermic cutaneous T-cell lymphoma. This technique is very potent in induction of apoptosis in T-lymphocytes, and modification of lymphocyte cytokine production.

Knobler et al. posed a hypothesis that reinfusion of ECP-treated lymphocytes induces autovaccination against the pathogenic T-cell clones[35].

Conclusion

Despite the introduction of numerous effective systemic medications and biologic agents in the field of dermatology, phototherapy remains a reliable, and often preferred, option for several dermatoses. UV light has major local and systemic immunosuppressive effects. Immune suppression, alteration of cytokine expression, and cell cycle arrest are the major contributors to the suppression of disease activity. Still, the actual pathways underlying phototherapy in most dermatoses are not known in details. Therefore, further studies on both clinical and basic photoimmunology will be needed to reach these goals.

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MEHANIZMI DJELOVANJA FOTOTERAPIJE U LIJEČENJU NAJČEŠĆIH BOLESTI KOŽE

S A Ž E T A K

Fototerapija označava upotrebu ultraljubičastog (UV) svjetla u liječenju nekoliko bolesti kože. Fototerapija koristi različite valne duljine ultraljubičastog zračenja. Trenutno, fototerapija obuhvaća širokospektralnu UVB (290–320 nm), uskospektralnu UVB (311–313 nm), 308 nm excimer laser, UVA 1 (340–400 nm), psoralen plus UVA (PUVA) i ekstrakorporalna fotokemoterapija (fotofereza). Međudjelovanje različitih fotobioloških mehanizama za sada nije u potpunosti razjašnjeno. Veliki broj bolesti kože ima dobar terapijski odgovor na fototerapiju, a neke od najvažnijih indikacija su: psorijaza, atopijski dermatitis, T-stanični limfom kože, cirkumskriptna sklerodermija i vitiligo. UVB zračenje prvenstveno djeluje na strukture epidermisa i papilarnog dermisa, dok UVA zračenje djeluje na papilarni i retikularni dermis, osobito na krvne žile. UVB zračenje apsorbiraju endogene kromofore, poput DNA jezgre, čime se potiče cijeli niz biokemijskih reakcija. Apsorpcija UV zračenja od strane DNA dovodi do stvaranja fotospojeva koji dovode do smanjene sinteze DNA. Pored ovog učinka, UV zračenje dovodi do otpuštanja prostaglandina i citokina koji imaju važan učinak u supresiji imunološkog odgovora. UV zračenje smanjuje broj Langerhansovih stanica, T limfocita i mastocita u dermisu. UV zraČenje također djeluje na molekule u citoplazmi i na staničnoj membrani. Najvažniji mehanizmi fototerapije su apoptoza, imunosupresija, te promjena ekspresije i sekrecije citokina. PUVA je oblik fotokemoterapije kod kojeg se koriste psoraleni i UVA zračenje. Psoraleni stvaraju unakrsne veze s DNA, inhibiraju replikaciju DNA i uzrokoju zaustavljanje staničnog ciklusa. Nakon izlaganja UVA zračenju psoraleni mijenjaju ekspresiju citokina i njihovih receptora. Osim navedenog, psoraleni djeluju na RNA, te ostale strukture stanice i putem slobodnih kisikovih radikala utječu na oštećenje proteina i lipida. PUVA snažno utječe na supresiju limfocita s različitim efektom na različite podvrste T limfocita. Psoraleni i UVA zračenje potiču i melanogenezu. Ekstrakorporalna fotofereza je metoda koja se primjenjuje u liječenju eritrodermijskog stadija limfoma kože. Ova metoda snažno potiče apoptozu T limfocita. Unatoč primjeni različitih učinkovitih sistemskih i bioloških lijekova u dermatologiji, fototerapija ostaje pouzdana terapija izbora u liječenju različitih bolesti kože.