

New applications of UVA-1 cold light therapy Polderman, M.C.A.

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Marloes C.A. Polderman

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Polderman, Marloes Christina Abichael

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New applications of UVA-1 cold light therapy

Proefschrift

ter verkrijging van de graad van Doctor aan de Universiteit Leiden, op gezag van de Rector Magnificus Dr. D.D. Breimer, hoogleraar in de faculteit der Wiskunde en Natuurwetenschappen en die der Geneeskunde, volgens besluit van het College voor Promoties te verdedigen op woensdag 26 april 2006 te klokke 15.15 uur

door

Marloes Christina Abichael Polderman

geboren te Leiden in 1971

Promotiecommissie

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Immanuel Kant (1783)

Aan Joost en mijn ouders

List of abbreviations

ANF	Anti nuclear factor
anti-dsDNA	Anti-double stranded DNA
anti-scl70	Anti-scleroderma 70
anti-Sm	Anti-Smith
anti-SSA/SSB	Anti- Sjögren's syndrome A/B
ATP	Adenosine triphosphate
CI	confidence interval
DASI	Dyshidrotic Area and Severity Index
DLQI	Dermatology Life Quality Index
ELISA	Enzyme-linked immunosorbent assay
EMR	Electromagnetic radiation
FAD	Flavin adenine dinucleotide
FASL	FAS-ligand
FMN	Flavin mononucleotide
ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
LP	Lichen planus
LFA-1	Lymphocyte function-associated antigen-1
MED	Minimal erythemal dose
MMP	Matrix metalloproteinase
MOS SF36	Medical Outcome Study 36-item short-form
NAD(H)	Nicotinamide adenine dinucleotide, oxidized form (reduced form)
NADP(H)	Nicotinamide adenine dinucleotide phosphate, oxidized form (reduced form)
PBMCs	Peripheral blood mononuclear cells
RIA	Radio immuno assay
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
ROS	Reactive oxygen species
SCLE	Subacute cutaneous lupus erythematosus
SCORAD	Scoring atopic dermatits
SLAM	SLE Activity Measure
SLE	Systemic lupus erythematosus
SLEDAI	SLE Disease Activity Index
UVA-1	Ultraviolet A-1
VAS	Visual analogue score

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Chapter 1

General introduction

Synopsis/Outline

UVA-1 therapy is a relatively new phototherapeutic modality. In this chapter, its position in the history of phototherapy, its physical properties and its biological effects are discussed. The objectives of this thesis are outlined at the end of this chapter.

UVA-1 in the history of phototherapy

The remedial use of sunlight has a long history. Egyptian and Indian healers used application of psoralen-containing plant extracts on the skin in combination with exposure to sunlight to heal leukoderma (vitiligo).¹ Around 400 BC Greek athletes were recommended to sun-bathe before their competitions. According to Hippocrates, exposure to sunlight would activate 'body resources' and restore 'dyscrasia' of the four body juices: yellow bile, black bile, phlegma, and blood.² However, too much sun exposure was considered to cause disturbance of the well-regulated movement of fluids by thickening, resulting in 'constipation' instead of 'purgation'. As an early form of photoprotection Plinius (23-79 BC) recommended to put the white of an egg on the face during sun-bathing.³ In the Middle Ages a white skin was fashionable. It proved that one belonged to the distinguished upper class, while a tanned skin identified the working class man. Consequently, heliotherapy (helios= sun) was not much used in that time.

It was not until the end of the nineteenth century that real interest in phototherapy returned. Niels Finsen from Denmark developed light therapy for the treatment of lupus vulgaris (cutaneous form of tuberculosis), for which he received the Nobel Prize in 1903. From then on, the development of phototherapy accelerated. However, the real 'boom' in phototherapy started in the late '70s. In 1974 Parrish *et al.* showed that ultraviolet A (UVA) irradiation of the skin preceded by orally administered 8-methoxypsoralen was very effective in the treatment of psoriasis. This new therapy was the first form of photochemotherapy and became known as PUVA (psoralen and UVA radiation).⁴ Around the same time, also broad spectrum ultraviolet B (UVB) was shown to be able to clear several types of psoriasis. A decade later, a new type of lamps with an emission spectrum consisting of a narrow peak around 311/312 nm (narrow-band UVB) was added to the phototherapeutic arsenal.¹ In that same period, Mutzhas *et al.* reported on new equipment emitting UV radiation in the 340-400 nm range.⁵ They used this long-wave UVA, later named "UVA-1", successfully for provocation of polymorphic light eruption (PLE) and photopatch testing. It proved to be less effective in the treatment of acne and vitiligo. Little more had been heard of this UVA-1 radiation until 1992, when this UV source was shown to be successful in the treatment of atopic dermatitis.⁶⁻⁸ At present, high dose (130 J/cm²) and medium dose (50 J/cm²) treatment schedules are used in UVA-1 therapy for atopic dermatitis and other dermatoses.

Physical properties of UV-radiation

The existence of invisible rays of sunshine was not acknowledged until 1800, when infrared and UV-radiation were discovered. Until then, Newton (1669) considered light to consist of small particles. A decade later, Huygens (1677) formulated the theory that light consisted of waves, like waves of water. This was much later supported by Hertz (1888), who showed that any electromagnetic radiation (EMR) consists of waves, but that no medium is needed for its propagation. In 1905 Einstein proved light to be a discontinuous sequence of small energy states (photons) and not visible matter. It was not until the second half of the last century that quantum physics were able to combine these two theories into a single "Theory of light". Nowadays, sunlight is defined as electromagnetic radiation (EMR), consisting of photons with varying, wave-length dependent, energy levels.⁹

According to wavelength, and accompanying physical and biological characteristics, the electromagnetic spectrum can be divided into gamma radiation, X-rays, UV radiation, visible light, infrared radiation, and electrical/radio waves (Fig. 1.1). The solar spectrum consists of UV, visible, and infrared radiation, but only 3-7% of solar radiation energy reaching the surface of the earth is UV radiation. This radiation can be subdivided into vacuum UV (10-200 nm), UVC (200-290 nm), UVB (290-320 nm), and UVA (320-400 nm). Vacuum UV-radiation derives its name from the fact that these wave-lengths are absorbed by oxygen and consequently not transmitted through air. UVC is almost totally absorbed by the (intact) ozone layer. UV radiation that reaches the earth essentially consists of UVB and UVA, the biologically most active components.



Figure 1.1. Subdivision of electromagnetic radiation

Physical properties of UVA-1 radiation

Recently, UVA-1 (340-400 nm) has been distinguished from the rest of the UV spectrum for its different qualities and distinctive therapeutic potential.⁵⁻⁸ The longer wave-length of UVA-1 penetrates deeper into the skin and is therefore able to reach the deeper layers of the dermis and possibly the subcutis. In contrast, UVA-2 and UVB can penetrate only the upper layers of the dermis.^{10,11} These differences in penetration depths are in conflict with the differences in the energy levels:

The equation:
$$E = hc/\lambda$$
,

in which E is energy, h is Planck's constant (6.63 x 10^{-34} J/s), c is the speed of light in meters per second and λ is the wave-length in meters, shows that the longer wave-lengths of UVA-1 contain lower energy when compared with UVA-2 or UVB. One would expect that radiation with higher energy would penetrate deeper in the skin. However, the ability of UVB, UVA-2, and UVA-1 to penetrate the skin is principally determined by the concentration of UV absorbing compounds in the skin. There are much more UVB than UVA absorbing molecules in the epidermis, which is the reason why UVA (and especially UVA-1) radiation can reach the deeper layers of the skin.

Different UVA-1 cabins have slightly different emission spectra, which may account for different treatment results. For most of our studies we used a BioSun Med UVA-1 cold-light unit (BioSun Sylt-Service, Wennigstedt/Sylt, Germany) (Fig. 1.2.). The apparatus emits photons with wavelengths of 340-550 nm and the usual irradiance was around 30 mW/cm². Owing to a special filter system that eliminates all heat producing infrared radiation and a

ventilation system providing a cool breeze, this UVA-1 therapy is also called UVA-1 cold light therapy. From the same company we used a Photomed hand-UVA-1 unit (BioSun Sylt-Service, Wennigstedt/Sylt, Germany) to treat patients with dyshidrotic hand eczema (Chapter 3).



Figure 1.2. BioSun Med UVA-1 cold light unit

Biological effects of UVA-1

The biological effects of UV radiation are the consequence of the absorption of photons by molecules in the skin, so-called chromophores. These chromophores may transform into new molecules, called photoproducts. Some photoproducts are removed by repair mechanisms, others affect signal transduction pathways or are toxic to the cells. The resulting biological

effects can be visible within minutes (as in solar urticaria), hours (sunburn), or it may take days (e.g. activation of subacute cutaneous lupus erythematosus), or even years (photoaging) before they are discernible.

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Different wave-lengths are absorbed by different chromophores. The effects of UVA-1 absorption by its chromophores (Table 1.1) are not yet fully known.

Table 1.1. UVA-1 chromophores (see list of abbreviations)

UVA-1 chromophores:
Pyridine (NAD/NADH, NADP/NADPH)
Riboflavin (FAD, FMN)
Porphyrin
Tryptophan
Pteridine (folic acid)
Urocanic acid ¹²
Cobalamin (vitamin B ₁₂)
Beta-carotene
Bilirubine

However, there is strong evidence that UVA radiation is an oxidizing component of sunlight that exerts its biological effects mainly by producing reactive oxygen species (ROS).^{13,14} The ROS production is based on photosensitizing properties of some absorbing compounds. Well-known examples of natural photosensitizers are porphyrins and riboflavins, which after UV absorption in the presence of oxygen, produce singlet oxygen ($^{1}O_{2}$) and the superoxide radical (O_{2}^{-}). The latter is converted by the enzyme superoxide dismutase to hydrogen peroxide. The concentration of UVA-1 absorbing photosensitizers is highest in mitochondria and so it is obvious that these organelles are very sensitive to UVA-1 radiation. The most important of the presently known effects of UVA-1 on the different cutaneous cell types and their clinical implications, are described in detail below, and are summarized in table 1.2.

UVA-1 effect on keratinocytes

Not much is known about the biological effects of UVA-1 on epidermal keratinocytes. Several experiments suggest that UVA-1, although not as much as UVB, can lead to thickening of the epidermis.¹⁵⁻¹⁷ After 60 J/cm² UVA-1 (*i.e.* >1,5 MED) on 3 consecutive days in 12 healthy subjects, a mean epidermal thickening of 11% was observed, compared with 25% increase of epidermal thickness after 1,5 MED of UVB.¹⁶ This observation is supported by results of a cell-cycle study in mice. In these experiments, comparably erythematogenic doses of UVB and UVA-1 resulted in more cycling cells after UVB than after UVA-1,¹⁵ accounting for more pronounced epidermal hyperplasia after UVB than after UVA-1. Another explanation for epidermal thickening is provided by UV(B) induced small proline-rich protein 4 (SPRR4) which improves the epidermal integrity after UV exposure and prevents skin desquamation.¹⁸

The intercellular adhesion molecule-1 (ICAM-1), expressed on the surface of keratinocytes is a cytokine-inducible adhesion molecule. It serves as a receptor, to which the leucocyte adhesion molecules lymphocyte function-associated antigen-1 (LFA-1 = CD11a/CD18 integrin on leukocytes) and Mac-1 (= CD11b, α chain of integrin on macrophages) are able to bind.^{19,20} In this way ICAM-1 plays a role in the induction and maintenance of epidermal inflammatory infiltrates.²¹ Whereas normal skin is practically devoid of ICAM-1 expression on keratinocytes,²² expression of this molecule is found to correlate with the degree of inflammation in psoriasis and atopic dermatitis.²²⁻²⁴ After successful UVA-1 therapy for atopic dermatitis, ICAM-1 expression on keratinocytes was significantly reduced.²⁵ Downregulation of ICAM-1 expression by keratinocytes most probably results from decreased levels of IFN- γ produced by skin infiltrating Th1 cells. A direct effect of UVA-1 is

Cell type	Riological effects	Theraneutical implications
Keratinocytes	Epidermal hyperplasia	Atopic dermatitis
	IL-10 production \uparrow^{20}	
	ICAM-1 expression ²⁵	
Langerhans cells	Decreased numbers ²⁷⁻²⁹	Atopic dermatitis ^{28,31}
)	Antigen presenting cell function ²⁷	1
	CD80/CD86 expression ³⁰	
Melanocytes	Increased (human) or decreased (mice) numbers ^{32,33}	
ì	Melanin production ³²	
T cells	Apoptosis ^{13,14,34,35}	Atopic dermatitis, ^{7,31} sclerotic skin diseases, ³⁶
	IFN-y production $L^{21,25}$	cutaneous T cell lymphoma, ³⁷⁻⁴⁰ lichen planus ⁴¹
Eosinophils	Decreased numbers ^{42,43}	Atopic dermatitis ⁴²
ſ	Eosinophilic cationic protein ⁴²⁻⁴⁴	
Mast cells	Decreased numbers ^{45,46}	Urticaria pigmentosa ⁴⁶
	Urinary histamine J ⁴⁵	9
Fibroblasts	Matrix metalloproteinases (MMPs) (MMP-1, -2, and -3) $^{47-}$	Sclerotic skin diseases, like localized
	53	scleroderma, ⁵⁶⁻⁵⁸ and graft vs. host disease ^{59,60}
	Collagens I and III ⁵⁴)
	Elastic fiber content J ⁵⁵	
B cells	Immunoglobulin production (Chapter 7)	SLE ⁶¹⁻⁶⁴
Endothelial cells	VEGF production \uparrow^{36}	Angiogenesis in sclerotic skin diseases ³⁶

Table 1.2. Main effects of UVA-1 on different cutaneous cell types and their therapeutical implications

unlikely, particularly since UVA-1 irradiation of normal keratinocytes *in vitro* leads to singlet oxygen mediated ICAM-1 upregulation.⁶⁵

Next to a possible anti-inflammatory effect through the reduction of ICAM-1 expression on keratinocytes, UVA-1 has been shown to enhance mRNA levels of the anti-inflammatory cytokine IL-10 in human keratinocytes *in vitro*.²⁶ However, this UV induced anti-inflammatory effect has never been confirmed on protein level *in vivo*.

UVA-1 effect on Langerhans cells

Various authors have reported that UVA-1 may affect epidermal Langerhans cells. In a paper by Dumay *et al.*,²⁷ epidermal cell suspensions prepared from skin biopsies, taken three days after exposure to a single dose of UVA-1 (30 or 60 J/cm²) contained decreased numbers of Langerhans cells. Furthermore, a downregulation of antigen presenting cell function was seen, which could partially be prevented by prior application of a sunscreen.²⁷ However, other data report that UVB, but not UVA-1 is capable of diminishing antigen-presenting cell function by interfering with the upregulation of CD80/86 molecules on Langerhans cells.³⁰ Furthermore, after UVA-1 irradiation a decrease of Langerhans cell dendricity, rounding up of the cell body, mitochondrial membrane alterations and reticulo-endothelial dilation was observed, apart from a dose-dependent reduction of epidermal Langerhans cells in the skin of atopic dermatitis patients was seen.²⁸ None of the mentioned studies specifies whether the reduction of Langerhans cell numbers results from apoptosis or from migration. Together, these changes result in an impaired immune response, but are advantageous in the treatment of atopic dermatitis.³¹

UVA-1 effect on melanocytes

Although observed in many of our patients, pigmentation of the skin resulting from UVA-1 therapy is not frequently reported of in literature. Homogeneous hyperpigmentation was described in humans, after repetitive and single UVA-1 irradiations in 2 studies.^{32,66} Skin biopsies taken from these volunteers after a single UVA-1 irradiation showed increased numbers of epidermal melanocytes and enhanced melanin production.³² However, others noticed an increase of melanocytes in pigmented hairless mice after a single erythemal dose of UVB radiation, but not after even high doses of UVA-1.³³

Apart from an effect on melanocyte numbers a shift of epidermal melanocytes towards the dermis was observed.⁶⁶ Some of these melanocytes exhibited fibrillar degeneration, others were morphologically intact. Fibrillar degeneration with consequent apoptosis can be considered a reaction to subtoxic cell damage.⁶⁶

As can be seen, not much is known about the effect of UVA-1 on melanocytes. More research needs to be done in this field.

UVA-1 effect on T cells

The dermal inflammatory infiltrate in patients with atopic dermatitis mainly consists of CD4-positive T-lymphocytes. These CD4-positive T-lymphocytes are also referred to as T-helper cells and can be subdivided in Th1 and Th2 cells according to their cytokine profile. Th1 cells mainly produce pro-inflammatory interferon gamma (IFN- γ), whereas Th2 cells are characterized by interleukin-4 (IL-4), IL-5, and IL-10 production.

Grewe *et al.* observed that clinical improvement of atopic dermatitis was associated with a reduction of increased levels of IFN- γ mRNA.²⁵ They also described that exposure of long-term cultured normal human keratinocytes to UVA-1 radiation caused an induction of

IL-10 mRNA expression and IL-10 protein secretion.²⁶ IL-10 in turn, inhibits the production of IFN- γ by Th1 cells, which among other things, leads to decreased ICAM-1 expression on keratinocytes.²¹ As discussed earlier, ICAM-1 plays a role in the induction and maintenance of epidermal inflammatory infiltrates.

Apart from the effect on T cell function and cytokine production, UVA-1 may also induce apoptosis of T helper cells.^{14,34} *In vitro* experiments have shown that UVA-1 induced T cell apoptosis is mediated by the generation of singlet oxygen and superoxide anions, as well as by increased FASL surface expression.^{13,14} Singlet oxygen is able to open mitochondrial megachannels, releasing apoptosis initiating factor and cytochrome *c*.³⁵ The latter leads to activation of caspase pathways, which is followed by apoptosis. Additionally, the activation of the FAS/FASL system in T cells leads to receptor-triggered apoptosis. FASL binds to FAS, thereby stimulating a signaling pathway leading to apoptotic death of of the FAS expressing cell. Through depletion of T cells in the dermal inflammatory infiltrate UVA-1 is thought to be effective in the treatment of various skin diseases with T cell involvement like atopic dermatitis,^{7,31} cutaneous T cell lymphoma,³⁷⁻⁴⁰ lichen ruber planus,⁴¹ sarcoidosis,^{67,68} granuloma annulare.⁶⁹ or pityriasis lichenoides.⁷⁰

UVA-1 effect on eosinophils

UVA-1 has been shown to be able to lower the increased numbers of peripheral eosinophils and serum levels of eosinophilic cationic protein (ECP) in patients with atopic dermatitis.^{42,44} As serum levels of ECP were proposed as a marker of disease activity,⁷¹ this effect could contribute to the positive effect of UVA-1 in the treatment of atopic dermatitis. The effect of UVA-1 on eosinophils is not only useful in atopic dermatitis. Plotz *et al.* observed improvement of skin lesions and relief of itching in patients with hypereosinophilic syndrome, accompanied by reduction of peripheral eosinophil numbers and ECP.⁴³ Recently, we have successfully used UVA-1 therapy in several patients with eosinophilic cellulitis (unpublished observation). The mechanism by which UVA-1 radiation generates its effect on eosinophils is unknown.

UVA-1 effect on mast cells

Immunohistochemical experiments show that the dermal mast cell is another potential target cell for UVA-1.²⁸ A decrease of mast cell numbers was observed after high-dose UVA-1 therapy (130 J/cm²) in the skin of patients with atopic dermatitis²⁸ and after both high- and medium-dose (60 J/cm²) UVA-1 therapy in cutaneous mastocytosis.^{45,46} An *in vitro* study showed that increasing doses of UVA-1 inhibited histamine release from human mast cells (HMC1 cell line).⁷² Patients with urticaria pigmentosa reported relief from itching, diarrhea, and migraine with normalization of histamine in 24-hour urine after high-dose UVA-1 therapy.⁴⁶ After both high- and medium-dose UVA-1 therapy, pruritus and quality of life improved significantly.⁴⁵

UVA-1 effect on fibroblasts

Considering UVA-1 irradiation easily reaches the dermal part of the skin,¹¹ dermal fibroblasts are another obvious target. Several *in vitro* and *in vivo* studies have shown a UVA-1 induced increase of interstitial matrix metalloproteinase (MMPs, *i.e.* MMP-1, MMP-2, MMP-3) mRNA^{47,48} and protein⁴⁷ in human fibroblasts of healthy volunteers, morphea patients,⁴⁹ and patients with systemic sclerosis.^{50,51} Mempel *et al.* have revealed a decrease of collagens I and III in skin biopsies of patients with atopic eczema after medium dose UVA-1 therapy.⁵⁴ As mentioned earlier, UVA-1 can generate reactive oxygen species (ROS).¹³ Since it has been

shown that both induction of oxidative stress and exogenously added H_2O_2 to human dermal fibroblasts lead to increased collagenase (MMP-1) mRNA levels *in vitro*^{52,53} it is most likely that oxygen species are mediators of the UVA-1-induced synthesis of matrix metalloproteinases.

The induction of collagenase, which degrades dermal collagen, may be an important mediator of photoaging (wrinkling)^{73,74} and may facilitate tumor invasion.⁵² Repeated suberythemal doses of (broad spectrum) UVA *in vivo* resulted in decrease of elastic fiber content, further contributing to photoaging.⁵⁵

The induction of collagenase can explain the effects of UVA-1 in the treatment of a number of sclerotic skin conditions, like localized scleroderma,⁵⁶⁻⁵⁸ scleroderma and acrosclerosis in patients with systemic sclerosis,^{50,75} sclerodermic type of graft versus host disease,^{59,60} scleredema,⁷⁶ and extragenital lichen sclerosus et atrophicus.^{77,78} UVA-1 mediated induction of other matrix-degrading enzymes, like proteoglycanase, leading to degradation of hyaluronic acid depositions is thought be responsible for the improvement of cutaneous lesions of patients with reticulate erythematous mucinosis (REM syndrome) after UVA-1 therapy.⁷⁹

UVA-1 effect on endothelial cells

Recently, it has been proposed by Breuckmann *et al.* that apart from T cell apoptosis and collagenase induction, UVA-1 phototherapy possibly has a third mode of action in patients with sclerotic skin diseases. The authors showed that UVA-1 phototherapy resulted in increased vascular endothelial growth factor (VEGF) expression, leading to increased vascularization.³⁶ This could also explain why we observed healing of therapy resistant

ulcerations in several patients with systemic sclerosis and in a patient with ulcerative sarcoidosis during UVA-1 therapy (unpublished observations).

Carcinogenic properties of UVA-1 radiation

The main short-term side effects of UVA-1 therapy are a minor erythema, tanning of the skin,⁶⁶ and slight xerosis cutis. As explained in one of the previous paragraphs, repeated UVA-1 therapy could very well lead to premature skin aging. Another, important long-term risk is a potential carcinogenic effect. Some decades ago, UVA was regarded to be noncarcinogenic.⁸⁰ Recent animal experiments have shown, however, that UVA-1 is able to induce skin cancer.^{81,82}

De Gruijl and coworkers accumulated many data on the induction of skin tumors by chronic UV exposure in albino mice. From these data they constructed an action spectrum for carcinoma induction. Maximum UV effectiveness for tumor induction was found to be at 293 nm (=UVB), with a steep decrease to the UVA area.⁸³ This striking difference in carcinogenicity between short- and long-wave UV radiation has been confirmed in various experimental situations. Indeed, repetitive exposure of healthy volunteers to 25 J/cm² UVA-1 resulted in nuclear p53 expression in epidermal keratinocytes,⁸⁴ indicating DNA damage. However, much lower p53 expression was found in human epidermis after 2 and 3 MED of UVA-1 than after 2 and 3 MED of solar simulated irradiation or 3 MED of narrowband UVB.^{85,86} In another study, transient p53 expression was detected in murine epidermis *in vivo* after 1 MED of UVA-1 (365 nm).¹⁷ This finding was confirmed by Beattie *et al.*⁸⁵ who showed that 3 MED of UVA-1 produced negligible numbers of sunburn cells in the epidermis

of volunteers, in contrast to 3 MED of narrowband UVB and 3 MED of solar simulated radiation. These apoptotic keratinocytes are thought to be other markers of DNA damage. So, there is accumulating experimental evidence that UVA-1 is less carcinogenic than UVB and UVA-2.

The difference in extent and type of carcinogenic outcome between UVA and UVB can be explained by their different wavelength-specific effects. UVB acts mainly through direct damage of DNA bases, leading to the formation of pyrimidine dimers, potential sources of mutations. UVA-1 irradiation, on the other hand, is not absorbed by DNA. Still it has been reported that it is capable of inducing pyrimidine dimers,⁸⁷ but approximately 10,000 times less efficiently than UVB and 100 times less efficiently than UVA-1 part of the spectrum the most important mechanism of DNA damage is based on the fact that reactive oxygen species, formed during photosensitisation of endogenous chromophores, may attack and damage DNA molecules.⁸⁸

Many researchers believe that UV radiation is strongly implicated in the etiology of cutaneous melanoma. However, the most harmful wavelength has not been identified with certainty. The well-known experiments with a pigmented fish model (Xiphophorus) provided the first indications that UVA radiation (and visible light) were effective in the induction of (fish) melanoma.⁸⁹ However, the results of two recent studies have suggested that UVB, and not UVA or UVA-1, plays an important role in the induction of melanomas. Only UVB-containing sources (isolated UVB and solar simulator) initiated melanomas in hepatocyte growth factor/scatter factor (HGF/SF) transgenic mice, whereas broad band UVA and a sunlamp, filtered to remove 96% of the UVB, did not.⁹⁰ In the second study, melanocyte proliferation in hairless mice was observed only after the irradiation with erythemal doses of UVA, but not after high doses of UVA-1.³³ However, there are also other data that show that

not only UVB (304 nm), but also UVA-1 (365 nm) was effective in causing an increase of melanocyte numbers in volunteers.^{32,66} So it seems that the role of UVA-1 in melanoma induction in humans still remains speculative.

In conclusion, although UVA-1 appears to be less genotoxic than the other parts of the UV spectrum, it is not harmless. It is very important not to underestimate its potential carcinogenic effects, particularly since the doses used for treatment are sometimes high, and because the long-term effects of UVA-1 irradiation are still unknow.

Objectives of the thesis

The main goal of the studies presented in this thesis was to examine the efficacy of UVA-1 therapy in several diseases characterized by the involvement of T and/or B cells. Whereas so far many reports have focused on working mechanisms of UVA-1 therapy in T cell mediated skin conditions, similar studies in SLE, a B cell mediated disease, are almost lacking. The second goal of our studies therefore was to clarify some of the mechanisms underlying the beneficial effects of UVA-1 therapy in SLE patients.

The majority of published data concern atopic eczema, in which efficacy of UVA-1 is beyond doubt. Some authors have reported good results with high-dose (130 J/cm², 3 weeks) UVA-1 in the treatment of atopic dermatitis,⁶ whereas others reported that also medium doses of UVA-1 (50 J/cm², 3 weeks) could be successfully applied.⁹¹ In order to better determine the place of UVA-1 in the dermatological therapeutic arsenal, its efficacy has to be compared directly with other treatment modalities and different UVA-1 treatment schedules must be evaluated. Tzaneva *et al.* observed that after the usual successful 3 weeks of medium dose

UVA-1 therapy, eczema relapsed relatively soon.⁹² To investigate whether the prolongation of treatment leads to a longer therapeutic response we treated 32 patients with atopic dermatitis with medium dose UVA-1 during 4 weeks and compared the clinical effect with the usual 3 weeks' schedule (29 patients) (Chapter 2). Considering the large impact of this disease on patients' quality of life, the effect of UVA-1 therapy on quality of life was also assessed.

The efficacy of UVA-1 therapy was also examined in patients with therapy resistant acrovesicular dermatitis of the hands (**Chapter 3**). The only report so far on positive effects of UVA-1 in the treatment of chronic dyshidrotic hand eczema regarded an uncontrolled study of 12 patients.⁹³ To confirm and expand these data we designed a controlled study in which UVA-1 therapy was compared with placebo therapy in 28 patients (**Chapter 3**).

The results of UVA-1 treatment of patients suffering from generalized lichen ruber planus and the effect on histopathological changes in the skin are reported in **Chapter 4**.

In the late 1980s, McGrath Jr *et al.* described a favourable effect of UVA radiation on SLE activity in a mouse model of SLE.⁹⁴ Later, they reported encouraging results in SLE patients treated with UVA-1.^{61,62} These results were unexpected, as photosensitivity is a frequently occurring symptom in SLE and patients are recommended to avoid sun light. In addition, sunlight or exposure to artificial ultraviolet (UV) lamps is believed to be capable of activating systemic disease in these patients.⁹⁵ Although the study designs had some shortcomings, the positive outcomes of the mentioned investigations encouraged us to design two studies to examine the efficacy of UVA-1 therapy in the treatment of patients with SLE. We treated 11 and 12 patients respectively, with 2 doses of UVA-1 in two double blind, placebo controlled, cross-over studies (**Chapters 5 and 6**). Two validated scoring systems were used in order to

evaluate disease activity in SLE patients during both trials. In addition, we studied the effect of UVA-1 exposure on auto-antibody titers and on quality of life.

In an attempt to elucidate the mechanism(s) behind the effects of UVA-1 in SLE, we performed an *in vitro* study which is described in **Chapter 7**. Questions addressed in this investigation concerned: (i) What percentage of UVA-1 actually reaches the dermis? (ii) Are peripheral blood mononuclear cells (PBMCs), and especially B cells, susceptible to UVA-1 induced cytotoxicity? and (iii) Has UVA-1 radiation effect on immunoglobulin production by activated B cells?

The results described in Chapters 2-7 are summarized and further discussed in **Chapter 8**. In addition, the position of UVA-1 therapy in the dermatological practice and some possibilities for future research are discussed in this chapter.

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Chapter 2

UVA-1 cold light therapy in the treatment of atopic dermatitis: 61 patients treated in the Leiden University Medical Center

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Abstract

Background: UVA-1 has been shown to be effective in the treatment of patients with atopic dermatitis. However, its optimal therapeutic conditions are not yet fully established. Methods: In an open prospective study we retrospectively compared the effect of 4 weeks therapy (32 patients) with the effect of the usual 3 weeks therapy (29 patients) in patients with atopic dermatitis, using a medium dose UVA-1 cold light (45 J/cm²), 5 days a week. Results: Scoring atopic dermatitis index (SCORAD) and dermatology life quality index (DLQI) quality of life indexes improved significantly during both 3 and 4 weeks UVA-1. Patients who were treated for 4 weeks showed a superior improvement of the SCORAD index (23.12 points, 95% confidence interval (CI) 16.09-30.16, vs. 13.32 points, 95% CI 5.61-21.04, p = 0.059), and the DLQI (5.41 points, 95% CI 2.38-7.88, vs. 3.86 points, 95% CI 1.88-5.84, p = 0.360), compared with patients who were treated for 4 weeks were treated for 3 weeks. However, the differences did not reach statistical significance. Only patients who were treated for 4 weeks were able to maintain their improvement 6 weeks after therapy. In both groups 50% of patients had intermittently used mild topical corticosteroids in the follow-up period.

Conclusion: Extension of UVA-1 therapy from 3 to 4 weeks results in a clinically relevant improvement of the outcome, and more prolonged therapeutic effects, measured by the SCORAD index.

Introduction

Since the 1970s psoralens and ultraviolet A radiation (PUVA) therapy has been successfully used for the treatment of atopic dermatitis. Alternative forms of phototherapy for the same disease have been UVB, narrow-band UVB and UVA-UVB combination therapy. In the 1980s UVA-1 treatment was introduced. This long-wave (340-400 nm) UVA treatment appeared to be a promising phototherapeutic modality. Some authors have reported on good results of high-dose (130 J/cm², 3 weeks) UVA-1 in the treatment of atopic dermatitis,¹ whereas others have shown that also medium doses of UVA-1 (50 J/cm², 3 weeks) could be successfully applied.² In several controlled trials, both high- and medium-dose UVA-1 proved to be more effective than UVA-UVB combination therapy.¹⁻⁴

We observed (Fig. 2.1.), together with some other authors that after a successful 3 weeks of medium dose UVA-1 therapy, eczema deteriorated relatively soon.⁵ To investigate if the prolongation of treatment leads to a longer therapeutic response we treated 61 patients with atopic dermatitis with medium-dose UVA-1 during either 3 or 4 weeks, and we evaluated disease activity, quality of life and duration of improvement after a follow-up period of 6 weeks.

Patients and methods

In an open prospective study 32 patients with atopic dermatitis were treated with UVA-1 during 4 weeks. Their therapeutic effect was retrospectively compared with the effect of UVA-1 in 29 patients who were treated during the usual 3 weeks. The mean age of patients was 33.4 years (range 17-73), 19 were male, 42 were female. The majority of the patients had skin type II (24/61), or III (29/61). The remaining eight patients had skin type I (4/61) or V

(4/61) Patients with moderate to severe atopic dermatitis [Scoring atopic dermatitis index (SCORAD) range 14.8-76.2] with insufficient effect of local corticosteroids, and no use of systemic corticosteroids or cyclosporine therapy in the previous 2 months, were included. A Photomed 250 000 unit (BioSun Sylt Service GmbH, www.biosunsylt.com), emitting photons with wave-lengths of 340-500 nm, with an irradiance of 31 mW/cm², was used. Owing to a filter system that eliminates all infrared (i.e. heat producing) radiation and a ventilation system providing a cool breeze, this UVA-1 therapy is also called UVA-1 cold-light therapy.

Patients were treated with 45 J/cm², 5 days a week, during 3 (29 patients) or 4 (32 patients) weeks. In the first week, the UVA-1 dose was increased from 3 J/cm² on Monday to 15 J/cm² on Tuesday, and further increased by 10 J/cm² every day to a maximum of 45 J/cm² on Friday. The cumulative UVA-1 doses were 573 and 798 J/cm² for the 3 and 4 weeks treatment schedule, respectively. During therapy patients wore goggles. Before the treatment, weekly during treatment, and 3 weeks and 6 weeks after treatment, two scoring systems were applied: the SCORAD (maximum possible score 103) ⁶ and the Dermatology Life Quality Index (DLQI, maximum possible score 30 = maximal discomfort).^{6,7} The examination of both scoring systems was performed by the same investigator who evaluated these parameters also in the 3 weeks' treated patients.

Except for the first week during which the daily dose was gradually increased to 45 J/cm², patients used no topical steroids or antihistamines until the follow-up. Emollients could be used infinitely until 3 h before irradiation to prevent glimmering of the skin and consequent radiation reflection. Temperature on the skin surface was measured after 10 min to compare with heat producing qualities of PUVA units reported in literature.⁸

A paired *t*-test was used to assess changes in the SCORAD index, and the DLQI during and after treatment. A non-paired *t*-test was used to compare mean changes between the 3 and 4

weeks treatment regimen. Analyses were performed according to the intention to treat principle. Statistical significance was defined as $p \le 0.05$.

Results

UVA-1 treatment resulted in a statistically significant decrease of the SCORAD index at the end of therapy [18.5 points, p = 0.0001, 95% confidence interval (CI) 13.26-23.67]. Baseline SCORAD (p = 0.75) and DLQI (p = 0.59) indexes of the 3 weeks' treated patients did not differ from those of 4 weeks' treated patients. The patients who had been treated for 4 weeks achieved better results (mean decrease of 23.12, SD = 19.52, p < 0.001, 95% CI 16.09-30.16) than those treated for 3 weeks (mean decrease of 13.32 points, SD = 20.28, p = 0.001, 95% CI 5.61-21.04) (Fig. 2.1.). However, this difference was just not statistically significant (p = 0.059, 95% CI - 20.00 - 0.40). Furthermore, when both groups had been treated for 3 weeks, the difference in improvement of the SCORAD index was not statistically significant (p = 0.256). After a 6 weeks' follow-up period 50% of patients were lost to follow-up in both groups. These patients had not responded better or worse to UVA-1 therapy than the patients who were not lost to follow-up. At that moment in time the SCORAD index of patients in both groups had increased by five points, which corresponded to a 21.6% loss of post-treatment effect for the 4 weeks' treated group and a 37.5% loss of post-treatment effect for the patients who were treated for 3 weeks. The patients from the 4 weeks treatment regimen still showed a significant improvement of their SCORAD index compared with pretreatment values, whereas those who were treated during 3 weeks did not (Fig. 2.1.). In both groups approximately 50% of patients had intermittently used mild topical corticosteroids during the follow-up period. The patients who did not need topical corticosteroids during follow-up had not responded better or worse to UVA-1 therapy than

the patients who did use local corticosteroids during follow-up. The DLQI showed a significant decrease after both 3 (3.86 points, SD = 5.20, p<0.000, 95% CI 1.88-5.84) and 4 weeks (5.41 points, SD = 7.53, p = 0.001, 95% CI 2.38-7.88) of UVA-1 therapy. The effect of two treatment regimens did not differ significantly (p = 0.360, 95% CI -1.81-4.90). Similar to the SCORAD index, only patients who had been treated for 4 weeks were still significantly improved at 6 weeks after therapy (Fig. 2.2.).

UVA-1 cold light therapy was generally well tolerated by patients. During treatment the maximum temperature at body distance was 34° C (range $24-34^{\circ}$ C). In PUVA-cabins temperatures up to 41^{\circ}C were reported.⁸ Some side-effects occurred. Fifteen (24.6%) patients experienced slight erythema in the first week that did not require any treatment and resolved spontaneously in a few days. This could be explained by the relatively light skin type of these patients (2/15 skin type I, 11/15 skin type II, 2/15 skin type III). Eight patients (13.1%) dropped-out: Two of them developed a photosensitive reaction (one had solar urticaria, the other probably had a light phototoxic reaction because of cosmetics), six others exacerbated after 1 (n= 2) and 2 weeks (n= 4). Their inferior therapeutic results might explain the large standard deviations of both SCORAD and DLQI improvements.

Discussion

With the use of medium doses of UVA-1 a mean improvement of SCORAD indices of 38% after 3 weeks was comparable with the results reported in literature.⁹⁻¹¹ A four weeks' treatment regimen appeared to result in a better outcome immediately after therapy than the 3 weeks' regimen. Although not statistically significant, the authors find the difference clinically relevant. Furthermore, compared with the 3 weeks' regimen, the maintenance of achieved clinical results during follow-up was improved. The 1-week extension of therapy might thus partly overcome the problem of deterioration of eczema after three weeks of medium dose UVA-1 as also reported by others.⁵ However, as both groups deteriorated five points during the 6 weeks' follow-up period, the authors realize that the improved maintenance of therapeutic results in the 4 weeks' treated group is partly explained by the superior improvement of the SCORAD and DLQI indexes immediately after therapy in the 4 weeks' treated group. The question remains whether the demanding treatment schedule, i.e. 5 days a week, is necessary and whether less frequent irradiations (2-3 /week) would have similar therapeutic effects.

PUVA therapy is a frequently used form of phototherapy in the treatment of atopic dermatitis. So far, there have been no studies published comparing the efficacy of UVA-1 with PUVA in the treatment of atopic eczema. Photosensitivity caused by psoralens requires protection of the eyes and the skin against sunlight during the rest of the day. Furthermore, up to 20% of patients suffer from side-effects of oral psoralens.¹² In our study, apart from some slight erythema in the first week and a photosensitive reaction in two patients, no short-term side-effects were seen.



Figure 2.1. Mean Scoring atopic dermatitis index (SCORAD) \pm standard deviation during 3 and 4 weeks UVA 1 and follow-up. 3 after/6 after: 3 and 6 weeks after UVA-1 therapy, *p=0.001, **p \leq 0.001.



Figure 2.2. Mean dermatology life quality index (DLQI) \pm standard deviation during 3 and 4 weeks UVA-1 and follow-up. 3 after/6 after: 3 and 6 weeks after UVA-1 therapy, *p = 0.001, **p \leq 0.001, #p = 0.015, ##p = 0.026.

PUVA and UVA-1 have different cellular targets. In PUVA therapy, psoralens bind to DNA molecules, followed by a UVA-induced photochemical reaction that is taking place in close vicinity of DNA molecules. Consequently, it is not surprising that long-term repetitive PUVA results in an increased risk of skin cancer.^{13,14} UVA-1 photons are not absorbed by nucleic acids. The most important targets of UVA-1 radiation are located in the mitochondria that contain relatively large concentrations of UVA-1 absorbing co-enzymes of the redox chain. DNA damage is mediated indirectly by the production of radical oxygen species. Although animal studies suggest that UVA-1 is less carcinogenic than UVA-2 and UVB,¹⁵ the long-term carcinogenic hazards of UVA-1 remain to be clarified and should not be underestimated. Some authors also showed that UVA-1 is capable of inducing squamous cell carcinoma in mice.^{16,17} This radiation can induce expression of p53 and pyrimidine dimers in human skin and in murine skin, however much less effectively than UVB and solar simulated radiation.¹⁸⁻²⁰ It is not yet clear whether UVA-1 plays a role in the etiology of melanoma. A recent experimental work has brought some evidence that UVB, but not UVA irradiation initiated melanoma in transgenic mice.²¹

UVA-1 radiation has been shown to generate singlet oxygen and superoxide anions.^{22,23} Extensive production of such reactive oxygen species can, apart from contributing to carcinogenity, in certain cell types, lead to apoptotic death.²⁴ Lymphoid cells have frequently been used for the investigation of UVA-mediated apoptotic responses because of their lower threshold for switching to the UV-induced apoptotic program.^{22,25} At least part of the therapeutic response to UVA-1 radiation could thus be ascribed to an apoptosis-inducing effect on the inflammatory infiltrate and especially on T-helper cells.^{23,26}

Our work supports the earlier studies of others showing that UVA-1 therapy can be successfully used as a monotherapy in the treatment of atopic dermatitis. For the prolongation of its therapeutic effects, a 4 weeks' treatment regimen is preferable to a 3 weeks' regimen.

Still, the place of UVA-1 in the treatment of atopic dermatitis needs to be better defined, e.g. by a multicenter comparative trial with other photo(chemo)therapeutic modalities.

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Chapter 3

A double-blind, placebo-controlled trial of UVA-1 in the treatment of dyshidrotic eczema

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Abstract

We carried out a randomized, double-blind, placebo-controlled study to examine the therapeutic effect of UVA-1 irradiation on dyshidrotic hand eczema. Twenty-eight patients were randomised to receive UVA-1 irradiation (40 J/cm²) or placebo, five times a week for 3 weeks. Evaluated by the DASI and the VAS, UVA-1 was significantly more effective after 2 and 3 weeks. Also, desquamation and area of affected skin improved significantly more after UVA-1. We did not find any difference regarding the response of patients with increased IgE blood levels (>100 IE/ml) compared with those having normal IgE concentrations. No side effects were observed. This study indicates that UVA-1 can cause a significant improvement of both objective and subjective signs of dyshidrotic eczema.

Introduction

Dyshidrotic eczema is a chronic symptomatic palmoplantar dermatitis. Frequently, patients do not respond properly to topical treatment and occasionally systemic corticosteroids are needed. Photo(chemo)therapy can be effective in dyshidrotic eczema, and in particular, PUVA has been reported to have some beneficial effect.¹⁻³ However, the use of psoralens is associated with increased carcinogenic risk. The absence of psoralen in UVA-1 therapy represents a significant advantage over PUVA. The first trial of UVA-1 in the treatment of chronic vesicular dyshidrotic eczema of the hands was reported in an uncontrolled study of 12 patients.⁴ As patients with dyshidrotic eczema may experience spontaneous remissions, efficacy of UVA-1 needed to be tested in a controlled manner. Here we describe the results of a double-blind, placebo-controlled study in which we examined the effectiveness of UVA-1 phototherapy.

Patients and methods

Patients

In the period of November 1999 until March 2001, 28 patients with dyshidrotic eczema of the hands were included in a randomized double-blind, placebo-controlled study after approval of the research project by the ethics committee of the hospital. Patients younger than 18 years and patients who used systemic immunosuppressive or immnunomodulating medication in the 2 months prior to participation were excluded. Other exclusion criteria were pregnancy and a history of UV-sensitivity or skin malignancy. Patients signed informed consent forms before participating in the study. They were randomly assigned to either UVA-1 (n=15) or placebo

treatment (n=13) by an independent investigator using a lottery system. A blinded investigator was responsible for the evaluation of the parameters.

The average duration of the patients' complaints was 8 years and 4 months (range, 4 months– 34 years). All had used potent topical steroids prior to the study, with little or no apparent benefit. There was no washout period for topical steroids. Seven patients had been successfully treated with PUVA in the past, but this had been delivered at least 6 months prior to UVA-1 therapy.

Irradiation equipment

A Photomed CL 3000 cold-light unit (Photomed World Industries, Hamburg, Germany, irradiance 60 mW/cm²) was used as hand irradiation equipment emitting photons with wavelengths of 340-500 nm. Owing to a filter system that eliminates all infrared irradiation and a ventilation system providing a cool breeze, Photomed UVA-1 therapy is also called UVA-1 cold-light therapy. Placebo treatment comprised of TL tubes, emitting visible light, covered with a blue plastic plate to mimic the blue UVA-1 light. During both treatments patients wore protective eyewear and their forearms were protected against scattered radiation.

Treatment schedule and evaluation

Patients were treated with 40 J/cm² UVA-1 or with placebo using the same irradiation time (11 min), 5 times a week for 3 weeks. The primary endpoint was the DASI (dyshidrotic area and severity index, maximum score 60). It consisted of the sum of the severity scores of vesicles (V), erythema (E), desquamation (D) and itch (I) (0 = none, 1 = mild, 2 = moderate, 3 = severe), multiplied by the surface of the affected area of the hand (A) (1=<20%,

to 5 = 81-100%).⁵ Secondary endpoints were a VAS (visual analogue score) for itch (maximum 10) and the separate items of the DASI. All parameters were determined before treatment, at the end of each week, and 3 and 6 weeks after treatment. Photographs were taken before and after 3 weeks of irradiation. Furthermore, we compared the effect of UVA-1 in non-atopic patients with that in atopic patients, the latter defined as those with increased IgE levels. During the entire treatment period patients used no topical steroids or antihistamines. No emollient was applied in the 3 h before irradiation.

Statistical methods

A paired *t*-test was used to assess changes in the DASI, its subscores and the VAS for itch during and after treatment. A nonpaired *t*-test was used to evaluate differences between the effect of UVA-1 and placebo treatment. Analysis was performed according to the intention-to-treat principle. Statistical significance was defined as p = 0.05.

Results

UVA-1 treatment resulted in a statistically significant mean decrease of the DASI of 6.5 points (SD 5.7) at the end of the second week and of 8.7 points (SD 6.7) at the end of the third week. Placebo showed a mean increase of DASI of 1.1 points (SD 7.3) and 0.4 points (SD 8.9) respectively. Difference between both regimens reached statistical significance at the end of the second and third week (p = 0.006 and p = 0.005, respectively) (Fig.3.1.). After therapy, there was also a significantly greater mean reduction of DASI subscores of desquamation (p = 0.005), itch (p = 0.005), and the affected skin area (p = 0.039) in the UVA-1 treated group when compared to the placebo treated patients. Although the mean DASI subscore of vesicles demonstrated





Figure 3.1. Changes in mean DASI score with standard deviations (SD) in patients with dyshidrotic hand eczema as a result of phototherapy with UVA-1 radiation and placebo light.

Table 3.1. Improvement of DASI, DASI subscores and VAS during 3 weeks of UVA-1 and placebo treatment.

	UVA-1 (n = 15)			Placebo $(n = 13)$		
Parameter	Mean (SD)	95% CI	Р	Mean (SD)	95% CI	Р
DASI	8.67 (6.72)	4,95-12,39	0,000*	-0,38 (SD 8,87)	-5,75-4,98	0,878
Desquamation	0.53 (0.74)	0,12-0,94	0,015*	-0,46 (SD 0,97)	-1,05-0,12	0,111
Itch	0.8 (0.68)	0,43-1,17	0,000*	-0,23 (SD 1,09)	-0,89-0,43	0,461
Affected area	0.6 (0.63)	0,25-0,95	0,003‡	0,08 (SD 0,64)	-0,31-0,46	0,673
Vesicles	0.73 (0.88)	0,24-1,22	0,006	0,69 (SD 1,32)	-0,1-1,49	0,082
Erythema	0.4 (0.91)	-1,0-0,9	0,111	0,08 (SD 0,86)	-0,44-0,6	0,753
VAS	2.31 (2.01)	1.16-3.42	0.001*	-1,37 (SD 4,05)	-3,82-1,08	0,26

*P = 0.005; *P = 0.039 when UVA-1 is compared to placebo. CI, Confidence interval.

a statistically significant reduction during UVA-1 (p = 0.006), there was no difference between UVA-1 and placebo. At the same time, there was a clear reduction (p = 0.005) in the mean VAS for itch in the UVA-1 group when compared to placebo (Table 3.1).

Nine patients had increased serum IgE (>100 IU/ml) levels. The four of them belonging to the UVA-1 group did not respond better or worse to UVA-1 than the patients with IgE serum concentrations within the normal range (p = 0.4). Four patients in the UVA-1 group who were previously successfully treated with PUVA did not respond better to UVA-1.

For ethical reasons some patients (mainly from the placebo group) could not be withheld from using topical corticosteroids after the 3 weeks of phototherapy. Six weeks after therapy the mean DASI in the UVA-1 treated group still showed a mean improvement of 10,85 points (SD 6,35). Although we could not properly evaluate the duration of the therapeutic effect, some patients probably need corticosteroid maintenance therapy to sustain the effect of UVA-1.

Apart from some minor erythemal reactions, no side-effects occurred. Three of the 13 patients in the placebo group prematurely discontinued therapy after 2 weeks because of exacerbation.

Discussion

UVA-1 radiation has been shown to be effective in the treatment of several skin diseases such as atopic dermatitis, localized scleroderma and mycosis fungoides.⁶⁻⁸ Grattan *et al.* found topical PUVA and UVA to be equally effective in the treatment of dyshidrotic eczema.¹ However, UVA-1 and UVA have the advantage that no psoralens, with their side-effects and increased carcinogenic risk, are used.

We have shown that UVA-1 is significantly more effective than placebo, and is very well tolerated. According to literature there are two main modes of action of UVA-1. One of them

is induction of apoptosis of lymphocytes in the inflammatory infiltrate through generation of reactive oxygen species⁹ and expression of FAS ligand on T lymfocytes.¹⁰ Lymphoid cells have frequently been used for the investigation of UVA-mediated apoptotic responses because of their lower threshold for switching to the apoptotic program.¹¹ Secondly, *in vitro* UVA-1 irradiation of cultured keratinocytes resulted in increased interleukin (IL)-10 mRNA expression and protein secretion.¹² As IL-10 is a Th-2 derived anti-inflammatory cytokine known to inhibit pro-inflammatory interferon- γ , this may explain the decrease in inflammation observed with UVA-1.

In addition, UVA-1 appears to have a lower carcinogenic risk than PUVA and UVB. Compared with solar simulator light, UVA-1 induced less photodamage (pyrimidine dimers) in murine skin.¹³ Likewise, in human skin 1 and 2 minimal erythemal doses from a solar simulator gave rise to twice the levels of p53 induced by UVA-1.¹⁴ In another study, UVA-1 also induced less tumour suppressor gene p53 than "broad" UVA.¹⁵ These observations indicate that UVA-1 causes less DNA damage. However, Lavker and coworkers have suggested that UVA-1 is capable of inducing dermal photo ageing.¹⁶

In conclusion, UVA-1 appears to be an effective therapy for dyshidrotic hand eczema, particularly on itch and affected area of skin. As no significant side-effects were observed, UVA-1 may constitute a promising therapy for an often recalcitrant skin disease.

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Chapter 4

Ultraviolet A1 in the treatment of generalized lichen planus: A report of 4 cases

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To the Editor:

Although it is considered to be self-limiting, lichen planus (LP) may exist for many years and may be generalized and difficult to treat. Four patients with histologically proven, therapy-resistant, generalized LP were treated with Ultraviolet A1 (UVA-1). None used medication known to improve LP or induce lichenoid drug reactions.

Treatment consisted of irradiation with 45 J/cm² for 5 days per week during two 4-week treatment periods with a 3-week interval, with the Photomed 250 000 (Photomed World Industries, Hamburg, Germany) emitting 30 mW/cm². Before and after treatment the affected body area, a 100-mm visual analogue score for itch and the Dermatology Life Quality Index (DLQI) were determined.¹

Case 1 was a 39-year-old woman who presented with a 4-month history of very itchy, generalized LP (Fig. 4.1a). Topical corticosteroids and retinoic acid had proven ineffective. After UVA-1 therapy 98% clearance was achieved (Fig. 4.1.b) and both itch and DLQI improved considerably. Thick plaques on her ankles resolved to thin patches. Histologically, all characteristic features of LP had normalized and only a sparse infiltrate was seen (Figs. 4.2a and 4.2b).

Case 2 was a 38-year-old man who presented with an 8-month history of hardly itching, generalized LP. Potent corticosteroid ointments were ineffective. After UVA-1 therapy, his LP had cleared for 88%. However, the patches on his ankles showed only some improvement. **Cases 3 and 4** were a 54-year-old father and his 17-year-old daughter who had a history of generalized LP of 22 and 9 years, respectively, and had little effect from topical corticosteroids and tretinoin cream. Long-term psoralen plus UVA (PUVA) therapy had previously been successful for the father, but his LP was exacerbated during a second PUVA course. UVA-1 therapy resulted in 82% clearance. The daughter had some temporary

improvement with UVB treatment 7 years earlier. In her case, UVA-1 therapy resulted in 41% clearance. The DLQI and the VAS improved in both. The thick patches on their ankles had not cleared completely.

In all 4 patients therapy-resistant LP lesions improved significantly (Table 4.1). In the past, PUVA therapy has also shown to be effective in the treatment of LP.² Biological effects of UV-rays are mediated by different photochemical mechanisms. UVA-1 radiation is known to generate singlet-oxygen and superoxide anions.³ Extensive production of such radicals can lead to apoptotic death of lymphoid cells⁴ that have been shown to have a lower threshold for switching to the apoptotic program.⁵ At least part of the therapeutic response to UVA-1 radiation may thus be because of an apoptosis-inducing effect on the inflammatory infiltrate.³ Whether other mechanisms also play a role in the therapeutic effect remains to be elucidated. UVA-1 therapy may be a promising additional therapy in the treatment of generalized LP, with no short-term side effects. Further studies with appropriate controls would be worthwhile.



Figure 4.1a Patient 1 before UVA-1 treatment



Figure 4.1b She was tanned after treatment and on this part of the body only marked hyperpigmentation was left where the LP lesions had been



Figure 4.2a Before UVA-1 the biopsy showed the characteristic histological features of LP



Figure 4.2b After UVA-1 some postinflammatory hyperpigmentation and a very sparse dermal infiltrate were seen

		Duration	% Body area affected		VAS		DLQI	
Patient No.	Gender/Age	of disease	Before	After (% reduction)	Before	After (% reduction)	Before	After (% reduction)
1	F/39 y	4 mo	79	2 (98)	14.8	0.3 (98)	6	2 (78)
2*	M/38 y	8 mo	65	8 (88)	0.3	0.2*	5	*0
С	M/44 y	22 y	27	5 (82)	2.7	0.7 (74)	L	1 (86)
4	F/17 y	9 y	22	13 (41)	5.2	4.2 (19)	7	5 (29)
DLQI, Dern	aatology Life Q	uality Index ((0-30); UVA-1, Ultravio	let A1; VAS, visual and	alogue sco	ore (0-10).		
*Patient 2 h	ad hardly any su	ubjective com	nplaints. Consequently, i	mprovement of these su	ubjective	parameters was not calc	ulated.	

Table 4.1. Patients' characteristics and results of UVA-1 treatment

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Chapter 5

UVA-1 cold light treatment of SLE: a double blind, placebo controlled crossover trial

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Abstract

Objective: Treatment of patients with systemic lupus erythematosus (SLE) often implies strong drugs with possibly serious side effects. Thus, there is a need for new immunosuppressive treatments. Long wave ultraviolet A (UVA-1) cold light therapy is an anti-inflammatory, immunomodulatory treatment with a possible systemic effect and few side effects. In the current study low dose UVA-1 cold light treatment was tested to determine whether it reduces disease activity in SLE.

Methods: Eleven patients with SLE were treated with UVA-1 cold light treatment and a placebo light treatment in a double blind, placebo controlled, crossover study. In two consecutive 12 week periods the patients were treated in the first three weeks with UVA-1 and placebo treatment or vice versa. The primary variables were the SLE Disease Activity Index (SLEDAI) and SLE Activity Measure (SLAM).

Results: The mean SLAM and SLEDAI showed a significant decrease of 30.4% (p=0.0005) and 37.9% (p=0.016) respectively after three weeks of UVA-1, and a non-significant decline of 9.3% (p=0.43) and 12.2% (p=0.54) respectively after three weeks of placebo treatment. In this small trial the difference in reduction of the disease activity indices during UVA-1 compared with during placebo treatment failed to reach the conventional border of significance (p=0.07). The total score of quality of life measure MOS SF36 did not improve significantly, but the subscore for vitality did improve.

Conclusion: Low dose UVA-1 cold light treatment was strongly suggestive of lowering disease activity in this double blind, placebo controlled study, and no side effects occurred.

Introduction

Current treatment of systemic lupus erythematosus (SLE) comprises non-steroidal anti-inflammatory drugs (NSAIDs), antimalarial drugs, prednisone, azathioprine, cyclophosphamide, chlorambucil, and methotrexate. These are drugs with potential side effects. Thus there is a need for alternative immunosuppressive treatments. Long wave ultraviolet A (UVA-1) cold light therapy is an immunosuppressive treatment^{1,2} with proven efficacy in patients with atopic dermatitis.³ The main short term side effects are a little sunburn and slight xerosis cutis. Although animal experiments suggest that UVA-1 is less carcinogenic than UVA-2 and UVB,⁴ the severity of long term side effects, like carcinogenicity and aging, is not yet clear. Compared with UVB (280-320 nm) and UVA-2 (320-340 nm), UVA-1 (340-400 nm) penetrates deeper into the skin, as far as the deeper layers of the dermis. Because of that deeper penetration the immunosuppressive and anti-inflammatory effects of UVA-1 are thought to be moderately systemic.

For a long time exposure to sunlight has been associated with exacerbation of SLE.^{5,6} Approximately 45% of patients with SLE are known to have photosensitivity.⁷ After exposure to sunlight patients show persistent erythema, erythematous papules or papulovesicles. Mainly UVB and, to a lesser extend UVA, are held responsible for the signs of photosensitivity occurring.⁸ Accordingly, the first reports on the beneficial effects of long wave UVA-1 in patients with SLE were unexpected.

In 1987 McGrath Jr *et al* described the favourable effect of UVA on SLE activity. Survival was prolonged only in irradiated mice in the New Zealand black/New Zealand white mouse model of SLE. Irradiated mice, compared with those not irradiated, had decreased anti-dsDNA levels and decreased spleen size at necropsy. Irradiation comprised wavelengths

predominantly in the UVA range (320-400 nm).⁹ Later, these authors also reported that low dose UVA-1 induced decreases of clinical disease activity, doses of systemic steroids, and autoantibodies in humans and improved disease activity scores during maintenance treatment when patients were irradiated twice a week for eight months after the initial three week treatment period.^{10,11} In 1993 Sonnichsen *et al.* published a case report about the successful treatment of a patient with subacute cutaneous lupus erythematosus with UVA-1.¹²

As UVA-1 irradiation may be promising in the treatment of SLE and as studies to determine the efficacy of UVA-1 in the treatment of SLE have been carried out by one research group only, we treated 11 patients with SLE in a double blind, placebo controlled, crossover study to compare results and establish a basis for further clinical and laboratory investigation.

Patients and methods

Patients

Eleven patients with mild to moderate SLE were included in this prospective study (Table 1). Patients (one male, 10 female) were recruited from the SLE outpatients' clinic of the rheumatology department. Their mean age was 38.1 years (range 18-56, median 35). Nine patients were white subjects, one was Surinam creole, and one was Indonesian. At entry their disease had a mean duration of 7.8 years (range 2-19, median 6). All patients fulfilled four or more American College of Rheumatology criteria for the diagnosis SLE and an SLE Disease Activity Index (SLEDAI¹³) of at least four. Patients were not allowed to change their drugs two months before entry. During the study, changes in drugs (except for NSAIDs) could only be made by the rheumatologist.

					SLEDAI	SLAM	SLEDAI	SLEDAI	SLAM	SLAM	
			Skin	Disease	at start	at start	during [‡]	during [‡]	during [‡]	during [‡]	
No	Age	Sex	colour	(years)	study	study	UVA-1	placebo	UVA-1	placebo	Drugs
-	35.0	ц	White	9	17	6	-	-0.5	ę	-2	Plaquenil 1x200 mg
7	29.2	Ц	White	7	12	12	6-	+2	-	- +	Plaquenil 2x200 mg, diclofenac 2x75 mg
ξ	27.5	Ц	White	9	13	17	4-	ς-	-9	ς	Ibuprofen 3x400 mg
4	48.6	Ц	Dark	3	12	15	-10	0	-2	-9	Plaquenil 1x200 mg, prednisone 1x10 mg, diclofenac 2x50 mg
5	30.9	Ц	White	8	16	13	-2	4-	-9	8+	Plaquenil 2x200 mg, prednisone 1x5 mg, naproxen 250 mg/week
9	18.4	ц	White	7	7	12	9+	- +	-	+2	Plaquenil 1x200 mg, prednisone 1x5 mg, ibuprofen 2x800 mg
٢	54.8	Ц	Dark	5	26	15	-19	9+	ς.	0	Prednisone 1x15 mg
8	34.9	Ц	White	19	14	16	-2	-2	8-	0	Plaquenil 1x200 mg
6	41.4	Ч	White	8	10	13	4-	-9	L-	-5	Prednisone 1x7 mg, naproxen 3x250 mg
10^{*}	42.0	Ц	White	17	12	15	6-	0	4-	- +	Plaquenil 3x200 mg, prednisone 1x5 mg, ibuprofen 2x400 mg
11*	56.2	М	White	5	20	17	L-	~	4	6-	Arthrotec 1x75 mg
*Pat	ients v	who rec	seived pl	acebo treati	ment first.						

[‡]Decrease (-) and increase (+) of SLAM and SLEDAI score during UVA-1 and placebo treatment.

Table 5.1. Patients' characteristics

Irradiation equipment

For UVA-1 irradiation the Photomed 250 000 (Photomed Medizintechnik GmbH Vertrieb Deutschland, Gehrden, Germany) was used. It emits photons with wavelengths longer than 340 nm. The instrument is equipped with a filter system that eliminates all infrared radiation, which significantly reduces heat production and increases comfort for the patients. Owing to these filters, the ventilation system that provides the patient with a cool breeze, and the blue color of the light, Photomed UVA-1 treatment is also called UVA-1 cold light treatment.

The placebo treatment comprised TL light tubes that could be placed under the UVA-1 light tubes. In this way patients used the same cabin for both treatments. To match the blue colour of the UVA-1 treatment, blue plastic covered the frame with the TL tubes. Patients could recognise differences between the lamps but they did not know which was the supposedly effective treatment. During both treatments patients wore protective eyewear.

Treatment schedule

The study had a double blind, placebo controlled, crossover design. During two consecutive 12 week periods patients were treated in the first 3 weeks. The following 9 weeks served as a wash out period. Patients were randomly allocated to group A (n=9) or group B (n=2) by an independent person. Irradiation consisted of total body irradiation with 6 J/cm², five days a week for three weeks or an equivalent time of exposure (3 minutes, 20 seconds) to placebo light. Group A was treated with UVA-1 for the first three weeks and was crossed over to be treated with the placebo light treatment in the second treatment period. Group B was treated with both UVA-1 and placebo light treatment in reverse succession. Irradiation was carried out during the winter months to minimise concomitant exposure to natural sunlight. Variables were evaluated every three weeks by the doctor until nine weeks after the last three week

treatment period. Both the doctor and the patients were blinded to the treatment throughout the study.

Our primary measures were two systems for clinical assessment of SLE activity, the SLE Disease Activity Index (SLEDAI)¹³ and the SLE Activity Measure (SLAM).¹⁴ The SLEDAI consists of 19 items representing nine organ systems. Each item is rated as present or absent. The SLAM includes 24 clinical manifestations for nine organ systems and eight laboratory variables to evaluate organs that cannot otherwise be assessed. All items are scored as 0 to 2, or 0 to 3 according to their severity. Two 100 mm visual analogue scales (VAS) accompany the SLAM score to measure the patient's and doctor's subjective ratings of disease activity. We decided to use the SLEDAI score because it discriminates single disease activity states among subjects well and completion costs little time. We included the SLAM score because it detects a treatment effect more sensitively.¹⁴ Furthermore, drugs were monitored and the patients filled in a validated quality of life questionnaire, Medical Outcome Study 36-item short-form health survey (MOS SF36),¹⁵ at each control visit. This quality of life questionnaire was rated in total as well as in separate scores for different features of quality of life: physical, social, and mental functioning, pain, vitality, and change in state of health. Apart from evaluation of clinical variables, titres of antibodies to SSA, SSB, Sm and RNP were determined as well as antinuclear antibodies and anti-dsDNA. Furthermore, a complete blood count, an erythrocyte sedimentation rate (ESR), and urine analysis were done every three weeks.

Statistical methods

McGrath found a 39% decrease (=6 points, SD 4.295, p<0.005) of disease activity scores in 10 patients who were treated with UVA-1.¹¹ To detect a decrease of six points when treated

with UVA-1 and 0 points when treated with placebo (SD 4.295) with a power of 80% (α =0.05, two sided tests) 11 patients were needed. A paired Wilcoxon test was used to determine changes in clinical and laboratory variables before and after UVA-1 and placebo irradiation and to determine significant differences (p<0.05) between improvement by UVA-1 and improvement by placebo treatment. All variables were evaluated for period and carry over effects.

Results

Of the 11 patients included in this study, none was lost to follow up. Clinical disease activity scores decreased more after three weeks of treatment with UVA-1 than after three weeks of placebo treatment. The mean SLAM showed a significant decrease of 30.4% (4.09 points, 95% confidence interval (CI) 2.49 to 5.69) after three weeks of UVA-1 by decreasing from 13.45 (=100%, SD (2.21)=16.43%) to 9.36 (=69.59%, SD (2.42)=17.99%) (p=0.0005) (Fig. 5.1.). Although the mean SLAM showed a non-significant decline of 9.3% (1.18 points, 95% CI -1.89 to 4.26) from 12.73 (=100%, SD (3.35)=26.32%) to 11.55 (=90.73%, SD (3.56)=27.97%) (p=0.43) after three weeks of placebo treatment, the SLAM did not decrease significantly more after UVA-1 than after placebo treatment (mean -2.91, 95% CI -6.39 to 0.57, p=0.07). Similarly, the SLEDAI decreased by 37.9% (5.55 points, 95% CI 1.24 to 9.85) after UVA-1 treatment and 12.2% (1.32 points, 95% CI -1.29 to 3.93) after placebo treatment, that is, from 14.64 (=100%, SD 5.12=34.97%) to 9.09 (=62.09%), SD 4.78=32.65%) after UVA-1 (p= 0.016) and from 10.82 (=100%, SD 5.78=53.41%) to 9.50 (=87.8%, SD 3.93=36.32%) after placebo treatment (p=0.54) (Fig. 5.1.). Again, the difference between decrease of SLEDAI after UVA-1 and after placebo was not significant (mean -4.23,



Figure 5.1. SLAM and SLEDAI showed statistically significant improvement during UVA-1. Improvement of these variables during placebo treatment was not statistically significant.

95% CI-10.11 to 1.65, p=0.07). Until six weeks after UVA-1 the decrease of SLAM and SLEDAI was significant compared with the SLAM and SLEDAI scores before treatment. Thus, the clinical effect of UVA-1 lasted for six to nine weeks. SLAM and SLEDAI scores did not show significant decreases immediately after, three, six, or nine weeks after placebo treatment.

The nine organ systems of the SLAM score were also evaluated separately. The score of integument (oral ulcers + cutaneous rash + vasculitis + alopecia) and the cardiovascular score (Raynaud + hypertension + carditis) showed significant improvement after UVA-1 cold light treatment in comparison with placebo treatment (p=0.04 and 0.03 respectively). These results were mainly due to improvement of rash (p=0.08) and Raynaud's phenomenon (p=0.06). Improvements in the other seven organ systems were not statistically significant.

Also, the MOS SF36 subscore for vitality improved more after UVA-1 (-15.91 points, from 33.64 to 49.55, 95% CI -29.58 to -2.24) than after placebo treatment (2.27 points, from 47.27
to 45.00, 95% CI -8.60 to 13.14) (p=0.03). Changes in the MOS SF36 total score and in the remaining subscores after UVA-1 were not statistically significantly different from changes in these scores after placebo treatment.

Four patients were anti-SSA positive. In these four patients a mean decrease of anti-SSA antibody titres of 2.75 U/ml was found after UVA-1 (from 87 to 84.25, 95% CI 3.25 x 10^{-2} to 5.47) and a very small mean increase of 0.25 U/ml after placebo treatment (from 83 to 83.25, 95% CI -3.26 to 2.76) was seen; the difference was not significant (p=0.06), however. Seroconversion from a positive to a negative anti-SSA status did not occur. Anti-dsDNA antibody status did not differ significantly throughout the study. Changes in the doctor's and the patient's VAS and changes in the complete blood count , the ESR, and the urine analysis after UVA-1 were not different from after placebo treatment. No changes in drugs were made. None of the evaluated variables showed a period or carry over effect.

Despite the fact that five of the 11 patients were known occasionally to be photosensitive, no signs of photosensitivity or other side effects occurred during UVA-1 or placebo treatment.

Discussion

Improvement of SLAM and SLEDAI during UVA-1 treatment was significant, whereas improvement of both scores during placebo was not. Although the small number of patients resulted in a p value of 0.07, we suggest that the better improvement of the SLAM and SLEDAI during UVA-1 compared with during placebo treatment has obvious clinical importance. We make this suggestion especially because UVA-1 has few short term side effects and certainly the side effect profile is better than that of most of the alternative treatments for SLE. Apart from improvement of SLAM and SLEDAI scores, which contain

both objective and subjective variables, objective serological monitoring of disease activity by evaluating titres of anti-SSA showed and obvious trend of improvement during UVA-1 treatment in the four anti-SSA positive patients included.

The statistically significant improvement of the integument and cardiovascular subscores of the SLAM and of the vitality subscore of the MOS SF36 quality of life index should be interpreted with some caution. It should be kept in mind that testing of subscores increases the risk of statistical significance by chance. One could correct by Bonferroni correction, though this method is considered to be conservative.

In a double blind, placebo controlled, crossover design McGrath *et al.* treated 26 patients with SLE with less favourable results.¹⁶ After UVA-1 treatment, group A showed a significant 1.7 point decrease of the SLAM from 8.4 (2.9) to 6.7 (1.9) (p<0.05). Decrease of the SLAM in group B after UVA-1 was not statistically significant. The lack of wash out periods in their study risked carry over effects. Furthermore, the authors did not evaluate placebo effects by comparing the changes in SLAM after UVA-1 with the changes in SLAM after placebo treatment. In an uncontrolled study McGrath treated 10 patients with 6 J/cm² UVA-1 (five times a week for three weeks).¹⁰ The treatment resulted in improvement of various clinical measures. However, these variables were not combined in a commonly used disease activity scoring system and were consequently not easily comparable with our results. Furthermore, a different type of UVA-1 lamp was used.

The working mechanisms of UVA-1 are largely unknown. In the treatment of atopic dermatitis UVA-1 light is used in much higher doses.³ Apoptosis of certain T cell populations, resulting from singlet oxygen generation, is believed to play a part in this therapeutic effect. Owing to the apparent risk of photosensitivity in patients with SLE, we used a very low dose of UVA-1. No signs of photosensitivity occurred in any of our patients. Symptoms of

photosensitivity are reported to occur in patients with SLE when irradiated with UVA doses higher than 20 J/cm²,⁸ and thus a UVA-1 dose higher than 6 J/cm² might result in a better outcome. Also, it is not known how long the clinical effect of UVA-1 in patients with SLE lasts once the treatment is stopped. In our trial the effect lasted for six to nine weeks. A maintenance treatment of one or two irradiations a week might possibly prolong clinical effectivity.

The working mechanism of UVA-1, the effect of a higher dose of UVA-1, and the effect of maintenance UVA-1 treatment in patients with SLE are currently under investigation.

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Chapter 6

Efficacy of UVA-1 cold light as an adjuvant therapy for systemic lupus erythematosus

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Abstract

Objective: The assessment of the efficacy of therapy of patients with moderately active systemic lupus erythematosus (SLE) with low doses of UVA-1 cold light.

Methods: A double blind, placebo-controlled, cross-over study design was used for the examination of the efficacy of low doses of UVA-1 radiation (12 J/cm²/day for 15 days) in 12 patients.

Results: UVA-1 treatment resulted in a significant decrease of well-validated disease activity index indexes [the SLE activity measure (SLAM) (p<0.001) and the SLE disease activity index (SLEDAI) (p=0.007)], whereas neither score improved significantly during placebo treatment. Furthermore, UVA-1 therapy proved to be more effective (mean decrease 4.8 points) than placebo [mean decrease -1.7 points (*i.e.* an increase)] when measured by the SLAM (p=0.001, 95% CI -7.56 to -2.28), but not by the SLEDAI. Two patients had transient skin reactions at the beginning of treatment.

Conclusion: UVA-1 therapy appears to be a useful adjuvant treatment modality for patients suffering from moderately active SLE. Its effect could possibly be explained by reduction of B-cell function or apoptosis of plasma cells.

Introduction

Systemic lupus erythematosus is an autoimmune disease characterized by the production of a large variety of autoantibodies by B cells, leading to inflammation in various organs.¹ Current therapies, such as glucocorticoids, azathioprine and cyclophosphamide, are effective, but their side-effects may account for considerable organ damage in the course of the treatment.²

One of the frequently occurring symptoms in SLE is photosensitivity. In addition, sunlight or exposure to artificial ultraviolet (UV) lamps is believed to be capable of activating the disease. Although the mechanisms of the photosensitive skin reaction and SLE activation may be different, both adverse effects of UV exposure are the reason why patients are recommended to avoid sun exposure.

For that reason, it was quite unexpected when McGrath Jr *et al.*³ described a favourable effect of UVA radiation on SLE activity in a mouse model of SLE. Later, McGrath Jr *et al.*^{4,5} reported encouraging results obtained in SLE patients treated with a long-wavelength fraction of the UVA spectrum (340-400 nm), called UVA-1. This part of UVA is known to have a positive immunomodulating effect in some inflammatory skin diseases.^{6,7}

We have recently reported on our first experience with whole-skin UVA-1 cold light treatment of SLE patients.⁸ Being aware of the risk of photosensitivity we originally exposed our patients to only 6 J/cm² of UVA-1, five times a week. After 3 weeks of exposure the disease activity indexes SLE Activity Measure (SLAM)⁹ and SLE Disease Activity Index (SLEDAI)¹⁰ were lower than at the beginning of the phototherapy. However, some minor placebo response was observed as well, which possibly explains the lack of statistical significance when the effect of UVA-1 on SLAM and SLEDAI was compared with that of placebo treatment. No side-effects occurred during or after treatment.

These first results encouraged us to set up a new controlled clinical trial with the use of higher doses of UVA-1 cold light.

Patients and methods

After approval of the ethical committee we treated 12 patients with moderately active SLE, according to the revised criteria for SLE of the American College of Rheumatology.¹¹ Patients with a minimal SLEDAI of 4, with no changes in therapy during the last 2 months and without discoid skin lesions were included after their written consent was obtained.

A BioSun Med CL 3000 cold-light unit (BioSun Sylt, Wennigstedt, Germany) was used for the irradiations. The apparatus emits photons with wavelengths of 340-500 nm. Owing to a filter system that eliminates all infrared irradiation and a ventilation system providing a cool breeze, this UVA-1 therapy is also called UVA-1 cold light therapy. Placebo treatment was carried out using a panel of thermoluminescent (TL) tubes covered with a blue plastic plate that could be inserted into the UVA-1 cabin, to mimic the blue UVA-1 light. Patients could recognize differences between the two treatments on account of the absence of the cool breeze and warmth during placebo therapy. However, they did not know which was the supposedly effective one. Patients were allocated by an independent investigator for total body irradiations with 12 J/cm² UVA-1 (n=6) or an equivalent time of total body exposure (6minutes 40 s) to placebo light (n=6), five times a week for 3 weeks. After a 9-week wash-out period the patients received the alternative treatment.

The primary parameters followed during the treatment were the SLAM and the

SLEDAI. Furthermore, the Medical Outcome Study Short-form 36 (MOS SF36)¹² was used to evaluate quality of life and autoantibody titres [antinuclear factor (ANF), anti-double-stranded

DNA (dsDNA), anti-SSA, anti-SSB, anti-ribonucleoprotein (anti-RNP), anti-Sm, anti-Scl70, anti-Jo-1] were measured. Apart from non-steroidal anti-inflammatory drugs (NSAIDs), patients were not allowed to change their medication during the whole trial period.

A paired *t*-test was used to assess changes in the SLAM, SLEDAI and the MOS SF36 and auto-antibody titers during both treatments. A non-paired *t*-test was used to evaluate differences between the effect of UVA-1 and placebo treatment. Analysis was performed according to the intention-to-treat principle. A power calculation showed that 11 patients were needed.⁸ All variables were evaluated for carry-over and period effects.

Results

Twelve Caucasian patients (10 women, 2 men, age 23–58 yr), with moderately active SLE were included. Their mean SLAM and SLEDAI at time of inclusion were 13,42 (range 8-23) and 13,33 (range 6-23) respectively. At enrolment, their therapy consisted of low-dose prednisone (5/12), azathioprine (6/12), antimalarial drugs (7/12) and NSAIDs (8/12) (Table 6.1.).

As shown in Fig. 6.1., UVA-1 treatment resulted in a statistically significant decrease of both SLAM and the SLEDAI at the end of the third week of therapy, whereas during placebo treatment neither score improved significantly. Furthermore, UVA-1 therapy (mean decrease 4.8 points) proved to be more effective than placebo (mean decrease –1.7 points, *i.e.* an increase) when measured by the SLAM (p=0.001, 95% CI -7.56 to -2.28) (Table 6.2.). Frequently improving components were arthritis (6/9), myalgia/myositis (5/7), dyspnoea (4/4), fatigue (4/11), headache (4/4), leukocyturia/ erythrocyturia (4/7) and blood pressure (4/4).

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Table	6.1. Pat	tients' char	acteristics										
F/M	Age	Duration	Medication (mg)	Therapy 1	SLAM	SLAM	SLEDAI	SLEDAI	Therapy 2	SLAM	SLAM	SLEDAI	SLEDAI
	I	SLE (yr)		1	before 1	after 1	before 1	after 1	1	before 2	after 2	before 2	after 2
ц	34	ς	AZ 4x50, NSAID	UVA-1	23	12	21	17	placebo	10	16	19	18
Μ	58	8	HY 1x200, NSAID	UVA-1	14	11	9	9	placebo	12	10	4	4
Ц	39	10	NSAID, PR 5	UVA-1	14	12	10	9	placebo	7	11	9	8
ĹŢ	44	21	AZ 2x50, HY 2x200, NSAID	UVA-1	16	14	20	8	placebo	16	17	12	12
Ц	42	14	CH 1x100, PR 10	UVA-1	14	6	8	8	placebo	12	٢	10	7
Ц	43	0,5	HY 2x200	UVA-1	8	0	10	9	placebo	6	9	10	10
ĹŢ	37	9	NSAID	placebo	8	6	16	15	UVA-1	12	9	16	14
Ц	23	ς	AZ 2x50, NSAID, PR 7,5	placebo	15	12	23	7	UVA-1	6	9	9	5
Ц	42	9	HY 2x200	placebo	6	10	9	10	UVA-1	4	4	0	0
Ĺ	43	10	AZ 3x50, HY 2x200, NSAID, PR 7,5	placebo	16	15	12	8	UVA-1	12	8	8	4
Σ	33	6	AZ 3x50, PR 10	placebo	10	11	9	7	UVA-1	12	9	6	9
Ч	33	11	AZ 1x50, HY 1x200, NSAID	placebo	14	16	22	23	UVA-1	14	5	19	6
PR = m	nosinhe.	16 ∆7= a7a	thionrine HV= hydroxychloroduine										

PR= prednisone, AZ= azathioprine, HY= hydroxychloroquine

Table 6.2. Decrease of parameters, during 3 weeks' UVA-1 and placebo treatment: values are given as mean (SD; 95% confidence interval; p-value)

Parameter	UVA-1, n=12	Placebo, n=12
SLAM	4.75 (-3.12; 2.76 to 6.72; 0.000)*	-1.67 (3.13; -2.15 to 1.82, 0.857)
SLEDAI	3.67 (3.82; 1.24 to 6.09; 0.007)	1.83 (5.93; -1.59 to 5.26; 0.264)
MOS SF36**	-19.04 (80.61; -70.26 to 32.18; 0.431)	-53.56 (133.98; -138.70 to 31.55; 0.193)
*n= 0 001 when UVA-	1 is compared to placebo treatment	

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**A higher MOS SF36 means improvement of quality of life.



Figure 6.1. The effect of 3 weeks' total body irradiation with UVA-1 cold light and placebo on SLE activity expressed in SLAM and SLEDAI scores. The results are expressed as means with standard deviations. NS= not significant.

SLAM and SLEDAI scores at the beginning of the first treatment period did not differ from the scores at the beginning of the second treatment period (p=0.096), nor were these scores before UVA-1 different from before placebo treatment (p=0.479).

There were no significant changes of the MOS SF36, the ESR, leukocyte and differential counts, and C3- and C4-levels during UVA-1 treatment. The anti-RNP titre in one patient decreased by 25 units (31%), the anti-SSA titer in another by 16 units (22%).

Photosensitivity may occur in patients with SLE when irradiated with UVA doses higher than 20 J/cm².¹³ Two out of seven of our patients, known to be photosensitive, experienced some slight problems at the beginning of UVA-1 therapy, which consisted of a transient facial erythema in one and a minimal activation of subacute cutaneous lupus erythematosus (SCLE) in the other. In the latter patient the dose was subsequently reduced to 6 J/cm² at the beginning of the second week and the skin changes slowly disappeared.

Discussion

Whereas the dose of 6 or 12 J/cm² of short-wavelength UV (UVB) would cause serious burns with many apoptotic cells in the superficial skin, the same dose of UVA-1 does not generate any visible macroscopic or microscopic changes in the epidermis or dermis. Since it is known that UVA-1 photons penetrate easily to the superficial dermis, one must consider the possibility that UVA-1 radiation, by generating oxidative stress, may affect the metabolism of B cells and/or T cells in the capillary network of the skin.

SLE is one of the autoimmune diseases where expanded numbers of plasma cells are present in the blood. Recent investigations have shown that the number and frequency of circulating CD27^{high} plasma cells is significantly correlated with SLE disease activity.¹⁴ We suggest that these cells may be (one of) the targets of UVA-1 and that the irradiation might be able to suppress B cell activity or induce apoptosis of circulating activated B lymphocytes in the dermal and subcutaneous capillaries, resulting in lowered autoantibody production and subsequently in reduced disease activity. Alternatively, the B cell/T cell interaction could be affected.

SLAM appeared to be more suitable than SLEDAI for evaluation of therapeutic results over a course of time.⁹ This could explain why UVA-1 therapy proved to be more effective than placebo when measured by SLAM, but not when evaluated by SLEDAI.

Our results show that UVA-1 irradiation is a safe, effective adjuvant treatment for patients with moderately active SLE.

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Chapter 7

UVA-1 radiation suppresses immunoglobulin production of activated B lymphocytes *in vitro*

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(Submitted)

Abstract

Previous studies have shown that low-dose UVA-1 total body irradiations were capable of improving disease activity in patients with systemic lupus erythematosus (SLE). We hypothesized that UVA-1-induced suppression of immunoglobulin production by activated B cells in the dermal capillaries could be (partly) responsible for this effect. Our experiments with donor skin demonstrated that approximately 40% of UVA-1 could penetrate through the epidermis. Irradiation of peripheral blood mononuclear cells (PBMCs) with 2 J/cm² of UVA-1 resulted in 20% cell death. This toxic effect could totally be prevented by preincubation of the cell cultures with catalase. This indicates that the generation of hydrogen peroxide plays a role in UVA-1 cytotoxicity. T cells and B cells appeared to be less susceptible to UVA-1 cytotoxicity than monocytes. With the use of a CD40-CD40L B cell activation method we measured immunoglobulin production after various doses of UVA-1 irradiation (0-2 J/cm²). The dosis of 2 J/cm² caused a significant decrease of IgM, IgG, IgA and IgE production under the conditions of IL-10 or IL-4 (IgE) stimulation. Although UVA-1 can cause apoptosis of B lymphocytes, we show that relatively low doses of UVA-1 radiation also affect the function of these cells. Both effects may be responsible for the observed improvement of disease activity in SLE patients.

Introduction

Systemic lupus erythematosus (SLE) is a relatively common, chronic disease characterized by the production of multiple antibodies. Although the pathogenesis of this multiorgan disease remains unclear, B lymphocytes are held largely responsible for the immune dysregulation that underlies the disease process.¹ A significant proportion of therapeutic strategies in SLE are based on decreased production or the selective removal of circulating autoantibodies.^{2,3} About ten years ago, long-wave (340-400 nm) UVA radiation, designated as UVA-1, was introduced as a potential therapeutic modality for SLE patients.⁴⁻⁷ The development of this new approach in the treatment of SLE was quite contrary to the conventional knowledge of UV radiation being harmful to most patients with lupus erythematosus. The discovery by McGrath Jr and co-workers⁸ that UVA radiation had a favorable effect on disease activity and survival in a mouse model of SLE gave the first impetus to research in this new area. Later, promising results of uncontrolled and controlled studies of UVA-1 therapy in SLE patients were published by the same author.⁴⁻⁷ Decreased disease activity scores, sometimes accompanied by lowered auto antibody titres, were reported. From this work it has also become clear that UVA-1, but not UVB or visible light, was responsible for the beneficial effects.

We have recently conducted two double-blind, placebo controlled crossover studies in 11 and 12 SLE patients, respectively, using so-called UVA-1 cold light irradiation equipment.^{9,10} Being aware of the risk of photosensitivity in SLE patients we applied a low dose (6 J/cm² in the first, 12 J/cm² in the second study) of UVA-1 radiation daily, 5 days a week for 3 weeks. Even though we used UVA-1 equipment different from the apparatus used by McGrath *et al*, we could confirm the beneficial effect of UVA-1 treatment on disease activity and the

absence of side effects in both studies. In four patients with anti-SSA antibodies decrease of titres was recorded after UVA-1 therapy in the first study.⁹ In the second study the anti-SSA titre of one patient and the anti-RNP titre of another showed a marked decrease.¹⁰

Whereas the same dose of short-wavelength UV light (UVB) would cause serious burns with many apoptotic cells in the superficial skin, UVA-1 in such a dose does not generate any macroscopic or microscopic changes in the epidermis or dermis. In the present work we show that UVA-1 photons penetrate easily to the superficial dermis which enables them to affect the function of circulating lymphocytes, monocytes and other cells in the capillary network of the skin. In addition, we have found evidence that one of the mechanisms underlying the beneficial effect of UVA-1 in SLE patients could be a suppression of antibody production in activated B cells.

Material and methods

Penetration of UVA-1 through the epidermis

Three pieces of normal Caucasian skin (skin type II-III) were received after cosmetic breast reduction. The skin was washed 3 times with phosphate buffered saline (PBS) and subcutaneous fat was mechanically removed with small scissors. Each piece of skin was cut into three smaller parts (approximately 1.5 x 1.5 cm) which were incubated overnight with 3 ml dispase solution (Life Technologies B.V. Breda, The Netherlands) in a Petri dish at 4°C. The next day, the contents of the Petri dishes were further incubated for 1 hour at 37°C for 1 hour, after which the dermis was separated from the epidermis with two small tweezers.¹¹ The epidermis was placed on a microscope cover glass (23 x 32 mm), washed with PBS to

remove the rest of the dispase solution and kept in a Petri dish with a small amount of PBS to prevent desiccation.

The small pieces of epidermis were put on cover glasses and placed on the aperture of an ultraviolet A-1 (UVA-1) measurement device (BioSun Sylt Service GmbH). The epidermal sheets were large enough to cover the opening of the measurement device completely. By varying the distance between the lamps and the cell cultures, three different irradiances of UVA-1 (23, 31 and 47 mW/cm²) were applied and the percentage of penetrated UVA-1 radiation was determined. A BioSun Med 500 000 UVA-1 cold-light unit (BioSun Sylt Service GmbH, Germany, www.biosunsylt.com) was used as a UVA-1 source for these penetration experiments. The same unit was used for the irradiation of SLE patients in our previous study.¹⁰ The irradiance measured behind an empty cover glass put on the device's aperture was considered as being 100% penetration. Each measurement was performed in triplicate.

Determination of UVA-1 toxicity on PBMCs in vitro

The toxic effect of UVA-1 radiation was determined by evaluating the viability of irradiated peripheral blood mononuclear cells (PBMCs) with the use of trypan blue exclusion (Sigma, USA). The cells of three healthy volunteers were isolated from heparinized blood by Ficoll-Hypaque density-gradient (ρ = 1.077 g/ml, Pharmacia Biotech, Uppsala, Sweden) centrifugation and cultured in 24-wells plates. The wells were exposed to 0.5-10 J/cm² of UVA-1 radiation. To one of every three wells catalase (Sigma, USA), in a final concentration of 20 units/ml, was added prior to irradiation. Twenty-four hours after exposure to UVA-1 10 µl of trypan blue solution was added to each well and the cells were transferred to counting chambers to be manually counted. Counts were performed in triplicate.

To detect differences in the susceptiblity to UVA-1 toxicity of the various cell populations of the PBMCs, the viability of UVA-1 irradiated CD3 positive (T cells), CD14 positive (monocytes), and CD20 positive cells (B cells) was determined. PBMCs were irradiated with 0, 0.5, and 2 J/cm² UVA-1. Twenty-four hours later, cell death in the different cell populations was identified by using propidium iodide and flow cytometric analysis.¹²

Effect of UVA-1 on immunoglobulin production

PBMCs were obtained from heparinized blood of six healthy donors by separation on Ficoll-Hypaque (ρ = 1.077 g/ml, Pharmacia Biotech, Uppsala, Sweden) density-gradient centrifugation.

PBMCs were cultured in T75 flasks (Greiner, Alphen aan den Rijn, The Netherlands), on a layer of γ -irradiated mouse fibroblasts transfected with human CD40L, or on nontransfected (control) mouse fibroblasts (L cells).¹³ They were maintained in Iscove's modified Dulbecco's medium with glutamax (IMDM; Gibco BRL, Breda, The Netherlands), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS; Gibco BRL), 100 IU/ml of penicillin and 100 µg/ml of streptomycin (Boehringer, Mannheim, Germany).

Recombinant human cytokine IL-4 (200 units/ml) or IL-10 (50 ng/ml, PeproTech, Rocky Hill, NJ) was added to the cultures to evaluate the effect of these cytokines on immunoglobulin production.

Fifty thousand PBMCs were cultured on a layer of 5000 γ -irradiated (70 Gy) feeder cells: L-CD40L cells or L cells. The cultures were carried out in triplicate in 96-well culture plates at 37 °C and 5% CO₂ saturation. The total volume (cytokines included) was 200 µl.

Half of the tissue culture plates were irradiated daily with 0.5 or 2 J/cm² during the first week (i.e. 5 irradiations). After correction for the absorption of UVA-1 by the culture wells, these

doses corresponded to exposure times of 12 and 48 seconds, respectively. The other half of the culture plates received the same doses of UVA-1 during the second week of incubation. All supernatants were collected on day 15. IgM, IgG, and IgA production resulting from all conditions was measured by enzyme-linked immunosorbent assay (ELISA)¹⁴, IgE production was determined by radio immuno assay (RIA)¹⁵. A paired *t*-test was used to evaluate differences between immunoglobulin production after 0, 0.5, and 2 J/cm² UVA-1 irradiation. Statistical significance was defined as p=0,05.

The experiments were repeated with and without catalase added to the culture wells 30 minutes prior to UVA-1 irradiation. These cultures were irradiated in the second week of incubation. IgM, IgG, and IgA production resulting from these conditions was measured. Again, a paired *t*-test was used to evaluate differences between immunoglobulin production after 0, 0.5, and 2 J/cm² UVA-1 irradiation, and to assess differences between catalase and non-catalase conditions.

Results

Penetration of UVA-1 through the epidermis

In order to obtain an estimate of the proportion of UVA-1 radiation that can reach the superficial dermis where blood capillaries are present, we investigated the penetration of UVA-1 through the epidermis. Despite increasing irradiances, the penetration of UVA-1 through the epidermis of donor skin remained constant for all skin pieces (Fig. 7.1). However, interindividual variations in UVA-1 penetration ranged from 25% to 50%. The average penetration calculated from a total of 27 measurements was $39 \pm 12\%$, which implies that approximately 60% of UVA-1 radiation was absorbed by the epidermis.



Figure 7.1. Mean penetration of three different irradiances of UVA-1 (23, 31 and 47 mW/cm^2) through three different pieces of epidermis from normal Caucasian persons (skin type II-III). The columns show means \pm SD.

Determination of UVA-1 toxicity on PBMCs in vitro

After we had determined that a considerable part of UVA-1 irradiation was indeed capable of reaching the dermal layers of the skin and its capillaries, we started studying the cytotoxic effect of UVA-1 on PBMCs *in vitro*. As determined by trypan blue exclusion, increasing UVA-1 doses resulted in an increasing portion of non-viable PBMCs (Fig. 7.2). Pre-incubation of the cells with catalase totally prevented the toxic effects of UVA-1 radiation, suggesting the involvement of hydrogen



Figure 7.2. The cytotoxic effect of UVA-1 on PBMCs, expressed as the mean percentage of dead PBMCs determined by trypan blue exclusion, after a single irradiation with 0.5-10 J/cm² of UVA-1 radiation, in the presence and absence of catalase (20 units/ml). The values are shown as means \pm SD.

peroxide in UVA-1 toxicity. The shape of the toxicity curve of UVA-1 (Fig. 7.2) created the impression of a stepwise increase of cytotoxicity. This might be explained by the selective death of different PBMC subpopulations with increasing doses of UVA-1. Flow cytometric analysis was used to find out whether there were differences in the sensitivity of different PBMC subpopulations to low doses of UVA-1 radiation. The proportion of viable CD20 positive cells (B cells) was constant at 0.5 and 2 J/cm² and slightly increased at 10 J/cm² (Figs. 7.3a and 7.3b), whereas the proportion of CD3 positive cells (T cells) slightly increased. As the proportion of viable CD14 positive cells decreased with increasing UVA-1 dose, monocytes seemed to be the most sensitive cells.



Relative cell numbers

Figure 7.3a. FACS analysis detecting B lymphocytes (CD20), T lymphocytes (CD3) and monocytes (CD14) of PBMCs, before and after the UVA-1 irradiation (10 J/cm²) (as used for the calculation of the data shown in figure 7.3b).



Figure 7.3b. The proportion of viable CD3 positive (T-lymphocytes), CD14 positive (monocytes), and CD20 positive cells (B-lymphocytes) twenty-four hours after irradiation with 0, 0.5, 2, and 10 J/cm² UVA-1, determined by flow cytometric analysis. The values are presented as means \pm SD.

Effect of UVA-1 on immunoglobulin production

Since the B cell population appears to remain relatively invariable after low doses of UVA-1 *in vitro*, we investigated whether these UVA-1 irradiations result in decreased immunoglobulin production by activated B cells *in vitro*. In order to examine the effect of UVA-1 radiation on immunoglobulin production in peripheral blood B cells, we used the well-established CD40L culture system.¹³ PBMCs were cultured on a layer of γ -irradiated mouse fibroblasts transfected with human CD40L, in the absence or presence of recombinant cytokines IL-4 or IL-10. In previous studies by the group of Banchereau, Rousset *et al*

showed that IL-4 is essential for IgE production and that IL-10 is a critical factor for B cell activation and subsequent IgM, IgG and IgA (but not IgE) production.^{16,17} In this culture system, B cell activation consists of a first period of proliferation (week 1) followed by a second period of differentiation and antibody production.¹³ Immunoglobulin production at the end of the first week of incubation was generally very low (not shown). In cultures of fibroblasts lacking CD40-L and in those without added cytokines immunoglobulin production at the end of the second week was also very low (see Fig. 7.4 for IgM).



Figure 7.4. IgM production in non-irradiated cultures, after 2 weeks incubation of CD40L positive fibroblasts and fibroblasts lacking CD40L, under IL-4 or IL-10 stimulated or nonstimulated culture conditions. Data are shown as means \pm SD.

The combination of CD40L with IL-10 resulted in significant production of IgM at this point in time. At day 15, IgE production was present in the IL-4 stimulated conditions, suggesting that isotype switching took place during the second week of incubation (not shown).

Daily UVA-1 irradiation in the first week did not affect immunoglobulin production in the supernatants at day 15. However, UVA-1 exposure of the cultured cells during the second

week of incubation resulted in a dose-dependent decrease of IgM, IgG and IgA production in IL-10 stimulated conditions and IgE in IL-4 stimulated conditions (Fig. 7.5).

To investigate whether the decrease of immunoglobulin production after UVA-1 can be prevented by catalase, we repeated some of these experiments in the presence and absence of catalase. A statistically significant dose-dependant decrease of immunoglobulin production was observed for all isotypes tested, confirming the results described above. However, there were no significant differences in immunoglobulin production between the conditions with and without catalase (Fig. 7.6).

Discussion

Our experiments demonstrate that approximately 40% of UVA-1 reaches the dermis where it may influence various components including the circulating cells in the capillaries. UVA radiation, even in a relatively low dose, appears to be harmful for some white cells. Our investigations show that a dose of 2 J/cm² UVA-1 caused around 20% death of PBMCs. This toxic effect further increased with rising UVA-1 doses. However, pre-incubation with catalase totally prevented this UVA-1 induced cell death, suggesting that generated hydrogen peroxide plays an important role in this UVA-1 induced toxicity.

Absorption of UVA-1 by its chromophores (like porphyrines or riboflavins) can lead to photosensitization reactions that result in production of reactive oxygen species, singlet oxygen and superoxide radicals. The latter can undergo dismutation to hydrogen peroxide.¹⁸ Since the highest concentration of the mentioned UVA-1 absorbing compounds is present in mitochondria, these organelles are likely to be the most UVA-1 sensitive cellular targets. Mitochondrial injury leads to decreased ATP production, which in turn influences



Figure 7.5. The inhibitory effect of 0.5 or 2 J/cm^2 UVA-1 on IgM, IgG, IgA and IgE production in supernatants of PBMC cultures activated with CD40L and IL-10, during the second week of incubation. Data are expressed as means \pm SD of the changes in the immunoglobulin production expressed in percentages.



Figure 7.6. The effect of added catalase on IgM, IgG and IgA production in supernatants of PBMC cultures activated with CD40L and IL-10, after 2 J/cm² UVA-1 during the second week of incubation.

many synthetic processes. Many ATP molecules are necessary for protein synthesis. One can expect that even minor oxidative damage of mitochondria in activated B cells could consequently lead to decreased protein (immunoglobulin) production. Reactive oxygen species can also lead to apoptosis of B cells through activation of the caspase pathway by cytochrome *c*. Singlet oxygen is able to open mitochondrial megachannels, releasing apoptosis initiating factor (AIF) and cytochrome c.¹⁹

According to Farber *et al.*,²⁰ B cells are more sensitive to hydrogen peroxide than T cells. In our FACS experiments the sensitivity of three PBMC types was as follows: CD14>CD20 and CD3 (Fig. 7.3). The B cell population consists of 60 % naïve cells and 40% CD27-positive memory B cells.²¹ Recently, Jacobi *et al.*²² showed that the number of circulating CD27^{high} plasma cells correlated with disease activity in SLE patients. It would be interesting to investigate whether there is a difference between the cytotoxic effect of UVA-1 on different B cell populations.

A dose-dependent decrease of immunoglobulin production was observed after UVA-1 radiation in the second week in the IL-10 or IL-4 stimulated conditions. IgE concentrations in the supernatants were substantially lower than IgM concentrations (not shown). This can be explained by the fact that CD40-CD40L binding with IL-4 stimulation results in B cell proliferation and IgE isotype switching, whereas CD40-CD40L binding with IL-10 stimulation not only gives rise to B cell proliferation and IgG and IgA isotype switching, but also to plasma cell differentiation with increased immunoglobulin production. Plasma cell differentiation primarily takes place in the second week of cell culture, which explains the fact that only very low immunoglobulin concentrations could be measured in both non-irradiated and irradiated conditions at day 8 (not shown).

Twenty percent of cell death in the PBMC population was observed 24 hours after exposure to 2 J/cm² UVA-1. However, immunoglobulin production following daily irradiations with the same dose of UVA-1 in the second week was more than 20% reduced. An impaired B cell function could be responsible for this difference, or the cumulative effect of daily irradiations resulting in more cell death could be the cause. In the latter situation, the favorable effect *in vivo* could be longer lasting.

In additional experiments the effect of catalase on immunoglobulin production was investigated. Again, a significant dose-dependant decrease of immunoglobulin production was observed. However, no significant effect of catalase could be discerned. This observation could possibly be explained by the fact that catalase removes hydrogen peroxide exclusively extracellularly. This enables it to prevent UVA-1 induced cell death by lipid peroxidation of the outer cell membrane, since hydrogen peroxide, in contrast with catalase, can penetrate the cell membrane. However, extracellular catalase apparently does not have any profound effect on the intracellular concentration of UVA-1 induced hydrogen peroxide.

Because the epidermis absorbs a considerable portion of UVA-1 irradiation, doses higher than 2 J/cm² are probably needed to reach a therapeutic effect. In our clinical studies, we utilized 6 and 12 J/cm². According to our penetration experiments, these doses would correspond to approximately 2.4 and 4.8 J/cm² of UVA-1 reaching the dermal capillaries. Therefore, the effects of the doses used in our *in vitro* experiments were relevant to the situation in our previous clinical trials.

In conclusion, we have found evidence that long-wave UVA radiation, after penetration of the epidermis, is able to lower the production of antibodies in activated B cells and plasma cells. This effect can (partly) explain the clinical improvement observed in SLE patients after UVA-1 therapy.

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Chapter 8

General discussion

Synopsis

In the studies presented in this thesis we investigated the effects of UVA-1 therapy in patients with atopic dermatitis, dyshidrotic eczema, generalized lichen planus and systemic lupus erythematodes. In this final chapter, the results of these and other studies on UVA-1 therapy in these four conditions, as well as reports of UVA-1 therapy in other T cell mediated skin disorders are discussed, and possibilities for future research are described.

Atopic dermatitis

UVA-1 has proven effective in the treatment of various T cell mediated diseases, of which atopic dermatitis has been studied most extensively. Some authors reported on good results obtained by high-dose (130 J/cm², 3 weeks) UVA-1 in the treatment of atopic dermatitis,¹ whereas others showed that also medium doses (50 J/cm², 3 weeks) were successful.² The latter appears preferable to minimize the risk of potential long-term side effects. In order to better determine the value of UVA-1 in the treatment of atopic dermatitis, not only different doses of UVA-1, but also different treatment schedules must be evaluated. Tzaneva *et al.* observed that after the usual successful 3 weeks of medium dose UVA-1 therapy, atopic dermatitis deteriorated relatively soon.³ For that reason we investigated whether prolongation of the prevailing treatment schedule from 3 to 4 weeks leads to a longer therapeutic result. Chapter 2 describes an open prospective study of 32 patients with atopic dermatitis who were treated with medium dose UVA-1 radiation (45 J/cm²), 5 days a week, during 4 weeks. Their therapeutic effect was retrospectively compared with that of medium dose UVA-1 therapy in 29 patients who were treated during the usual 3 weeks. Both the SCORAD (scoring atopic dermatitis) and the DLQI (dermatology life quality index) improved significantly during both
treatment schedules, but at the end of treatment the 4 weeks' regimen did not prove to be more effective than the 3 weeks' regimen. However, 6 weeks after cessation of therapy the patients from the 4 weeks treatment regimen still showed a significant improvement of their SCORAD and their DLQI when compared with pre-treatment values, whereas those who were treated during 3 weeks did not. We conclude that medium dose UVA-1 therapy can be used successfully as a monotherapy in the treatment of atopic dermatitis, with positive effects on both disease activity and quality of life. For the prolongation of its remission time, a 4 weeks' treatment regimen is preferable to a 3 weeks' regimen.

To determine the position of UVA-1 therapy in the treatment of atopic dermatitis, the efficacy of UVA-1 therapy should also be compared with other types of phototherapy commonly used in atopic dermatitis, such as UVA-UVB combination therapy, PUVA and narrow band UVB.⁴⁻⁷ Recent studies showed improvements of disease activity scores of 50% for UVA-UVB, of 80% for oral PUVA, of approximately 65% for bath-PUVA and narrow band UVB^{4.5,8} and clearance or near-clearance of disease, in 14 of 15 patients in one study and in 74% of patients in another study, after oral PUVA.^{9,10} In several controlled trials, both high-and medium-dose UVA-1 proved to be more effective than UVA-UVB combination therapy.^{1,2,11,12} In our patients, the SCORAD improved 49% in the 4 weeks' treated patients and 27% in those treated during 3 weeks. From the results from literature PUVA and narrow band UVB appear to be better than UVA-1. However, there have been no controlled studies comparing these phototherapeutic modalities so far.

All mentioned treatment modalities have advantages and disadvantages. Different UV therapies have different treatment schedules, which demand different efforts from patients. Approximately the same number of treatments is needed for best results of UVA-1, PUVA or narrow band UVB therapy. UVA-1 is more time-consuming than PUVA and narrow band UVB with irradiations 5 times a week, but it has a shorter treatment period of 4 weeks.

Although more time-consuming, our experience is that many patients find the UVA-1 therapy rather relaxing. They often bring their own music to listen to while lying on the bed in the UVA-1 cabin. A disadvantage of these UVA-1 beds is that during irradiations the shadow areas in the pubic area and on the sides are not sufficiently treated (unpublished observation). Consequently, this kind of UVA-1 cabin is less suitable for treatment of malignant skin disorders, like cutaneous T cell lymphomas, for which complete clearance on all sides of the body is essential. However, other UVA-1 cabins are comparable to the usual PUVA and UVB cabins, and require patients to stand up during therapy.

Another difference between the treatment options concerns the side effects. Photosensitivity, caused by psoralens in PUVA therapy, requires protection of both eyes and skin against sunlight during the rest of the day. Furthermore, up to 20% of patients suffer from gastrointestinal side effects of oral psoralens.¹³ In narrow band UVB therapy patients may burn more easily, compared with UVA-1 therapy. Apart from a slight erythematous reaction, no short-term side effects are usually observed during UVA-1 therapy. Other potential short-term side effects for all mentioned phototherapeutic options are induction of UV sensitive photodermatoses and herpetic infection. Possible long-term side effects are skin aging and development of cutaneous malignancies. Experimental studies (summarized in chapter 1) suggest that PUVA and UVB are more mutagenic than UVA-1. However, long-term follow-up studies to assess skin cancer risk in UVA-1 treated patients have not yet been performed.

Although to our opinion the arguments are in favor of UVA-1 in the treatment of atopic dermatitis, the pros and cons should be discussed with the patient, before deciding which therapy will be used.

Dyshidrotic eczema

Phototherapy also belongs to the standard treatment options of dyshidrotic eczema. Both oral, cream-, and bath-PUVA have been reported to have some beneficial effects.¹⁴⁻¹⁶ The first report on the successful use of UVA-1 in the treatment of chronic dyshidrotic eczema of the hands concerned an uncontrolled study of 12 patients.¹⁷ They reported 81% improvement of the dyshidrosis area and severity index (DASI). However, since the severity of dyshidrotic eczema tends to fluctuate and spontaneous remissions may occur, the efficacy of UVA-1 needed to be established in a controlled manner. In a double blind, placebo controlled study **(Chapter 3)** we investigated 28 patients with dyshidrotic eczema of the hands. The results showed a 52% decrease of the DASI, after 3 weeks UVA-1 therapy, whereas after placebo treatment the DASI had slightly increased. Thus, UVA-1 treatment proved significantly better than placebo therapy.¹⁸ In a recent study UVA-1 and PUVA therapy were equally effective.¹⁹ These results further support the efficacy of UVA-1 in the treatment of dyshidrotic eczema. Since UVA-1 seems to be less carcinogenic than PUVA we prefer UVA-1 in the treatment of dyshidrotic eczema.

Lichen planus

Lichen planus (LP) is the third T cell mediated condition we investigated. Although it is generally self-limiting, LP may exist for many years, may be generalized and difficult to treat. Usually, it occurs on a limited number of localizations, in which case topical treatment usually suffices. In generalized LP, local therapy is too laborious and frequently unsuccessful. Since the 1970s, beneficial effects of oral photochemotherapy (PUVA) and bath PUVA for both localized and generalized LP have been described.²⁰⁻²³ In these publications, clearly

defined evaluation parameters were usually lacking, making comparison of results difficult. Results were formulated as excellent, good, complete clearance or at least 50% improvement in most patients. Furthermore, there was only one small controlled study among them, concerning hemi-corporeal oral PUVA therapy in 10 patients,²⁰ and no randomized, controlled studies comparing different forms of light therapy have been published so far. In chapter 4 we described the favorable effect of UVA-1 therapy in 4 patients with therapy-resistant, generalized lichen ruber planus.²⁴ A controlled study was not possible, as the generalized form of LP is relatively rare. Patients were treated with 45 J/cm² for 5 days per week during two 4-week treatment periods with a 3-week interval. After UVA-1 therapy nearly complete clearance was achieved in 3 patients, and considerable improvement in one. However, the tenacious thick plaques on the ankles showed only moderate improvement. Both the visual analogue scores for itch and the DLQIs improved considerably in all. In one patient, biopsies were taken before and after therapy. Histopathologic results showed that at the end of treatment the characteristic features of LP had normalized and only a sparse infiltrate remained. Our results, although concerning a limited number of patients, support the efficacy of UVA-1 therapy in generalized LP.

SLE

It has been known for a long time that a large proportion of patients with systemic lupus erythematosus (SLE) is sensitive to sunlight²⁵⁻²⁷ Mainly UVB and, to a lesser extent UVA, are held responsible for this photosensitivity.²⁸ Consequently, the first reports of beneficial effects of UVA-1 in patients with SLE were rather unexpected. McGrath was the first to show clinical improvement in an uncontrolled study of 10 SLE patients.²⁹ This was later confirmed by a double blind, placebo controlled, crossover study in 26 patients.³⁰ Unfortunately, both

studies were flawed due to use of an inappropriate disease activity scoring system, lack of wash out periods risking carry over effects, and failing in correct evaluation of placebo effects. Despite the imperfect design, the clinical results appeared interesting enough to warrant another double blind placebo controlled study.

Being aware of the risk of photosensitivity we originally exposed eleven patients with SLE to only 6 J/cm² of UVA-1 and to the same number of minutes of placebo light (see Chapter 5). In two consecutive 12-week periods patients were treated with UVA-1 and placebo therapy respectively, or vice versa, followed by a 9 weeks' wash-out period. The primary variables, SLE disease activity index (SLEDAI) and SLE activity measure (SLAM) showed a significant decrease after three weeks of UVA-1, but not after three weeks of placebo treatment. Although the MOS SF36 subscore for vitality improved more during UVA-1 than during placebo therapy, the difference was not statistically significant.

Chapter 6 describes a second study in which we applied a higher dose of 12 J/cm² in the same study design. UVA-1 treatment resulted in a significant decrease of both SLAM and SLEDAI at the end of the third week of therapy, whereas neither score improved significantly during placebo treatment. Furthermore, when UVA-1 treatment was compared with placebo treatment, the decrease of SLAM was statistically significant. However, the decrease of SLEDAI was not.

Two patients in the second study with a history of photosensitivity, experienced transient skin reactions at the beginning of UVA-1 therapy, which consisted of a transient facial erythema in one and a minimal activation of subacute cutaneous lupus erythematosus (SCLE) in the other. In this latter patient the dose was subsequently reduced to 6 J/cm^2 at the beginning of the second week and the skin changes slowly disappeared.

In all four patients with anti-SSA antibodies decrease of titres was recorded after UVA-1 therapy in the first study. In the second study the anti-SSA titre of one patient and the anti-

RNP titre of another showed a marked decrease, suggesting immunomodulating effects of UVA-1 therapy.

Although the pathogenesis of SLE remains unclear, B lymphocytes are thought to play a major role in the immune dysregulation that underlies the disease process.^{23,31} A significant proportion of therapeutic strategies in SLE are based on decreasing the production or the selective removal of circulating autoantibodies.^{23,32,33} Based on this information, the known deep penetration of UVA-1 radiation, and the observed decrease of auto antibody titres after UVA-1 therapy, we formulated the following hypothesis: UVA-1 induces suppression of immunoglobulin production by activated B cells in the dermal capillaries, which could be (partly) responsible for the observed improvement of disease activity in patients with SLE. In chapter 7 is explained how this hypothesis was confirmed. In order to obtain an estimate of the proportion of UVA-1 radiation that can reach the superficial dermis where blood capillaries are present, we measured the penetration of UVA-1 through isolated epidermis using a UVA-1 measurement device. The average penetration was 39 %, which implies that a large part of a given UVA-1 dose is indeed able to reach the superficial dermis and affect the function of circulating lymphocytes, monocytes and other cells in the capillary network of the skin. The toxic effect of UVA-1 radiation was determined by evaluating the viability of irradiated peripheral blood mononuclear cells (PBMCs). A dose as low as 2 J/cm² UVA-1 caused around 20% death of PBMCs. This toxic effect further increased with rising UVA-1 doses. However, pre-incubation with catalase totally prevented this UVA-1-induced cell death, suggesting that generated hydrogen peroxide plays an important role in UVA-1 toxicity. Flow cytometric analysis showed that in comparison with CD20 positive cells (B cells) and CD3 positive cells (T cells), CD14 positive cells (monocytes) seem to be the cells most sensitive to UVA-1.

A dose-dependent decrease of IgM, IgG, IgA and IgE production was observed after UVA-1 radiation of PBMCs in a well-established CD40-CD40L B cell activation system with IL-10 or IL-4 stimulation. Twenty percent of cell death in the PBMC population was observed 24 hours after exposure to 2 J/cm² UVA-1. However, a 47%, 44%, 36% and 60% decrease of IgM, IgG, IgA and IgE production, respectively, was observed following daily irradiations of PBMC cultures with the same dose of UVA-1. It is very likely that UVA-1 irradiation causes not only B cell apoptosis, but also affects immunoglobulin production of the surviving B cells. In addition, the cumulative effect of daily irradiations may bring about more cell death and even more decreased immunoglobulin production.

Whereas pre-incubation with catalase totally prevented UVA-1-induced cell death, no convincing effect of catalase on immunoglobulin production could be discerned. This could possibly be explained by the fact that catalase removes hydrogen peroxide exclusively extracellularly. This enables it to prevent UVA-1 induced cell death by lipid peroxidation of the outer cell membrane, since hydrogen peroxide, in contrast with catalase, can penetrate the cell membrane. However, extracellular catalase apparently does not have any profound effect on the intracellular concentration of UVA-1 induced hydrogen peroxide.

The observed effect of UVA-1 on immunoglobulin production suggests that UVA-1 therapy could also be effective in the treatment of other auto-immune diseases, apart from SLE. A likely prerequisite for success in the disease in question is the presence of activated circulating B cells and plasma cells in dermal capillaries. However, not all auto-immune diseases have B cells or plasma cells that produce antibodies outside the spleen and lymph nodes. A relevant auto-immune disease could be Sjögren's syndrome, in which the presence of antibody-producing cells in the peripheral blood has already been demonstrated.³⁴ In the treatment of SLE it is important to realize that this disease can be activated by UV radiation. Furthermore, different UVA-1 lamps have different emission spectra. Treatment with lamps

emitting even very small amounts of UVB should be avoided, because this radiation could cause apoptosis of epidermal keratinocytes with consequent activation of the auto-immune process.

In conclusion, we have found evidence that long-wave UVA radiation is able to lower the production of antibodies by activated B cells and plasma cells. This observation can, at least partly, explain the clinical improvement observed in SLE patients after UVA-1 therapy.

UVA-1 for other T cell mediated skin diseases

Apart from the four (skin) diseases discussed before, there are several case reports and small uncontrolled studies reporting on the beneficial effects of UVA-1 therapy in various other T cell mediated skin disorders. The results of these studies are summarized in Table 1. The results of UVA-1 therapy in sclerotic skin diseases are of particular interest and are discussed in more detail below.

Sclerotic skin diseases

Since the mid-1990s several reports on the effect of UVA-1 on localized scleroderma (morphea) have been published. Induction of interstitial matrix metalloproteinases (MMP), especially collagenase (MMP-1) is held responsible for hydrolysis of collagen in the skin after UVA-1 therapy, leading to improvement of sclerotic skin diseases.³⁵⁻³⁷ Apart from collagenase induction and T cell apoptosis,³⁸ increased vascular endothelial growth factor (VEGF) expression and reduced apoptotic endothelial cell turnover may also contribute to amelioration of disease activity by improving vascularization.³⁹ In one study, after 30 exposures, high dose (130 J/cm², n=10) was superior to low dose (20 J/cm², n=7) UVA-1 therapy. High dose UVA-1 therapy resulted in obvious reduction and softening of sclerotic

plaques, decreased skin thickness measured by 20 MHz ultrasound, and decreased skin elasticity determined by elastometry.⁵⁴ Nevertheless, others reported complete clearance of 80% of the lesions in 10 patients after 24 irradiations with only 20 J/cm², 4 times a week for 6 weeks, and disappearance or marked improvement of 80% of sclerotic lesions in 18 out of 20 patients after 30 treatments with 20 J/cm².^{55,56}

Also in patients with systemic sclerosis, softening of skin lesions on forearms and hands, improved passive range of motion of hand and wrist joints, improved cutaneous elasticity, and healing of ulcerations were observed.⁵⁷⁻⁵⁹ Some small studies and case reports were published on the effect of UVA-1 on some other sclerotic skin diseases like extragenital lichen sclerosus, sclerodermic graft-versus-host disease of the skin, scleredema, keloid, and nephrogenic fibrosing dermopathy.⁶⁰⁻⁶⁶ Although we have only limited experience in UVA-1 treatment of patients with keloids and scleredema, we could not fully confirm the reported positive effects in these patients. PUVA therapy has been found effective in the treatment of both localized scleroderma and systemic sclerosis as well.⁶⁷⁻⁶⁹ To our knowledge, no studies comparing the effect of UVA-1 with PUVA therapy have been published so far.

	Reference	Z=	Dose	Results
Granuloma annulare	Muchenberger et. al. ⁴⁰	4	130 J/cm ² , 5/7, 3 wks	1/4 cc, 3/4 pc
Sarcoidosis	Graefe <i>et. al.</i> ⁴¹	1	130 J/cm ² , 4/7, 25 exposures	Considerable improvement
	Mahnke <i>et. al.</i> ⁴²	-	60 J/cm ² , 4/7, 50 exposures	cc
REM	Meeuwes <i>et. al.</i> ⁴³	1	90 J/cm ² , $5/7$, 18 exposures	SC
Grover's disease	Breuckmann <i>et. al.</i> ⁴⁴	1	50 J/cm ² , 6/7, 3 wks, 2/7, 3 wks	Nearly cc
Pityriasis lichenoides	Pinton et. al. ⁴⁵	8	60 J/cm ² , 5/7, max. 30 exp.	6/8 cc, 2/8 >75% improvement
Pityriasis rubra pilaris	Herbst <i>et. al.</i> ⁴⁶	1	100 J/cm ² , 5/7, 3 wks + 25 mg acitretin	Dramatic improvement
Cutaneous T cell lymphoma	Plettenberg <i>et. al.</i> ⁴⁷	З	130 (n=2), 60 (n=1) J/cm ² , 5/7, 16-20 exp.	cc, stage IA, IB CTCL
(CTCL)	von Kobyletzki et. al. ⁴⁸	-	60 J/cm ² , 5/7, 3 wks	cc, mucinosis follicularis
	von Kobyletzki <i>et. al.</i> ⁴⁹	1	60 J/cm ² , 5/7, 3 wks	Considerable improvement, large cell CTCL
	Zane <i>et. al.</i> ⁵⁰	13	100 J/cm^2 , $5/7$, until max. effect	11/13 cc, 2/13 pc, plaque (8), nodular (4), erythrodermic (1) MF
Cutaneous mastocytosis	Gobello <i>et. al.</i> ⁵¹	22	130 J/cm ² , 5/7, 2 wks (n=10)	Considerable improvement of itch in most patients
			60 J/cm ² , 5/7, 3 wks (n=12)	
	Stege et. al. ⁵²	4	130 J/cm ² , 5/7, 3 wks	Relief from itching, diarrhea, migraine
HES	Plotz et. al. ⁵³	б	50 J/cm ² , 5/7, 3 wks	Improvement itch, skin lesions, neuropathy, and GI complaints
M. Wells	(unpubl. observation)	7	45 J/cm^2 , $5/7$, 3 wks	Considerable improvement
cc= complete clearance, pc=	= partial clearance, REM=	Reticu	llar ervthematous mucinosis. HES= Hypered	sinophilic syndrome. Gl=gastrointestinal. MF=mycosis fungoides

Table 8.1. Case-reports and small, uncontrolled studies on the effect of UVA-1 on various diseases

Conclusion

UVA-1 radiation is a useful therapeutic option for various T cell mediated and sclerotic skin diseases. We think that UVA-1 is the phototherapy of choice in atopic dermatitis and dyshidrotic eczema, and that this treatment could be a valuable therapeutic option in patients with generalized lichen planus and sclerotic skin diseases. To minimize potential carcinogenic risks, medium dose UVA-1 regimen are preferable to high dose regimen. Controlled studies are needed for further validation of the place of UVA-1 therapy in the dermatological practice. Interesting possibilities for future research comprise the effect of UVA-1 therapy in the treatment of auto-immune diseases, other than SLE.

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Summary

UVA-1 therapy is a relatively new form of light therapy. Since the beginning of the 1990s, the therapeutic effects of long wave UVA sources have been investigated in patients with atopic dermatitis. One of the mechanisms behind the positive effect of UVA-1 therapy in these patients is the UVA-1-induced apoptosis of T cells in the dermal inflammatory infiltrate. During the 1990s the number of indications for UVA-1 light therapy has substantially increased. Mostly T cell mediated conditions were investigated with the use of "low dose" (around 10 J/cm²), as well as "medium dose" (around 45 J/cm²), and "high dose" UVA-1 (around 130 J/cm²).

In **chapter 2**, the investigation of the efficacy of medium dose (45 J/cm²) UVA-1 in patients with atopic dermatitis is described. Since it is known that after the customary treatment schedule of 15 irradiations during 3 subsequent weeks deterioration of atopic dermatitis can occur relatively soon, we investigated if prolongation of therapy with one week will result in better clinical outcome. Both the SCORAD (scoring atopic dermatitis) and the DLQI (dermatology life quality index, a validated quality of life score) improved significantly during both the 3 and the 4 weeks' treatment regimen. There was no difference between both groups. However, 6 weeks after therapy the patients from the 4 weeks' treatment regimen still showed a significant improvement of their SCORAD and their DLQI compared to pre-treatment values, whereas those who were treated during 3 weeks did not.

In **chapter 3** successful UVA-1 therapy in patients with dyshidrotic eczema is described. The effect of UVA-1 therapy was investigated in a double blind, placebo controlled study. UVA-1

therapy proved to be significantly better than placebo therapy, adding dishydrotic eczema to the T cell mediated indications for UVA-1 light therapy.

Lichen ruber planus, a T cell mediated condition as well, usually occurs only on the predilection places, in which case it is treated topically. However, if it manifests itself as a generalized skin disorder, local therapy is impractical. In **chapter 4** we described the favorable effect of UVA-1 therapy in patients with chronic, generalized lichen ruber planus. Moreover, this chapter documents the toxic effect of UVA-1 monotherapy on the cells that form the typical dermal inflammatory infiltrate. UVA-1 therapy can be a good alternative for this extensive form.

Since UVA-1 has proven to be effective in the treatment of dermal T cell mediated conditions, it is to be expected that UVA-1 could easily penetrate to the dermal capillaries and affect there circulating cells. Except for T cells, B cells could also be influenced. Systemic lupus erythematosus (SLE) is characterized by the production of auto-antibodies by activated B-lymphocytes, giving rise to inflammation in various organs. The usual therapeutic options involve systemic corticosteroids, azathioprine, and cyclophosphamide but can be accompanied by various, possibly serious side-effects.

On account of the well-known risk of photosensitivity, we initially investigated the effect of merely 6 J/cm² UVA-1, in a double blinded, placebo controlled, cross-over study (chapter 5). After 3 weeks, this treatment resulted in decrease of both the SLE activity measure (SLAM) and the SLE disease activity index (SLEDAI), two validated methods used for the determination of disease activity in SLE patients. Although the UVA-1 treatment resulted in the improvement of clinical scores, the difference between the UVA-1 effect and the placebo effect was not statistically significant.

To investigate whether a higher dose has a better effect we treated SLE patients with 12 J/cm² in a second study with a similar experimental design (chapter 6). After 3 weeks of 12 J/cm² UVA-1, both the SLAM and SLEDAI had significantly improved. Additionally, UVA-1 therapy proved to be more effective than placebo treatment, when measured by the SLAM. In both *in vivo* studies an effect on auto-antibody titers was observed. Apart from problems of temporary photosensitivity in some and slight activation of subacute cutaneous lupus erythematosus (SCLE) in one patient, no side effects occurred. With these controlled studies we confirmed the positive effects of UVA-1 radiation in the treatment of patients with moderately active SLE (chapter 5 and 6). The auto-antibody profile of the patients was very heterogeneous, which is often the case with SLE patients. Nevertheless, a clear decreasing trend was observed in the anti-SSA titers after UVA-1 treatment in the first study.

This observation and the decrease of the anti-RNP and anti-SSA titers of the 2 patients in the second study encouraged us to investigate the effect of UVA-1 on the immunoglobulin production by B cells *in vitro* (chapter 7). Toxic effect on activated B cells and plasma cells in the dermal capillaries could result in decreased immunoglobulin production with consequent improvement of disease activity in SLE patients.

First, we measured the UVA-1 penetration through the epidermis. It turned out that approximately 40% of UVA-1 could reach the dermal cells. Then, we wanted to find out how sensitive B lymphocytes were to UVA-1 cytotoxicity. Twenty-four hours after irradiation with rising doses of UVA-1 we found increasing numbers of PBMCs to be dead. Pre-incubation of the cultured cells with catalase, which breaks down hydrogen peroxide, prevented the cell death completely. Determination of UVA-1 cytotoxicity for different PBMC subpopulations showed that the monocytes were the most sensitive to UVA-1 radiation.

Finally, we investigated the effect of UVA-1 radiation on immunoglobulin production by activated B cells of healthy individuals. The IgM-, IgG- and the IgE production decreased with increasing doses of UVA-1, in IL-10 or IL-4 stimulated conditions. A similar effect on the IgA production was also visible, but did not reach statistic significance.

Although in the *in vitro* part 20% of PBMCs died after 6 J/cm² of UVA-1, this was not enough to fully explain the observed reduction of IgM and IgE production. We suggested that the toxic effect of UVA-1 can also cause a reduction of B cell function. We believe that the production of hydrogen peroxide plays an important role in these toxic effects of UVA-1 radiation.

In conclusion, UVA-1 can be effective not only in T cell mediated diseases, but also in B cell mediated conditions, like systemic lupus erythemadosus. It would be interesting to investigate the possibility of using UVA-1 therapy for other auto-immune diseases. However, a necessary condition for success would be that these diseases have activated B cells/plasma cells in their dermal capillaries.

Samenvatting

UVA-1 therapie is een relatief nieuwe vorm van lichttherapie. Sinds begin jaren 90 wordt het therapeutisch effect van de langgolvige UVA bronnen onderzocht bij patiënten met consitutioneel eczeem. De voornaamste verklaring voor het positieve effect van UVA-1 therapie bij deze patiënten is de UVA-1 geïnduceerde apoptose van T cellen in het dermale ontstekingsinfiltraat. In de loop van de jaren negentig is het aantal indicaties voor UVA-1 lichttherapie fors toegenomen. Veelal T-cel gemedieerde aandoeningen worden in deze periode onderzocht, waarbij zowel "low dose" (rond 10 J/cm²), "medium dose" (rond 45 J/cm²), als "high dose" UVA-1 (rond 130 J/cm²) wordt gebruikt.

In **hoofdstuk 2** hebben wij de werkzaamheid van medium dosis (45 J/cm²) UVA-1 bij patiënten met constitutioneel eczeem getoetst. Omdat na het meest gangbare behandelschema, van 15 belichtingen gedurende 3 weken, relatief snel een recidief van de klachten kan optreden, hebben wij onderzocht of verlenging van de therapieduur met 1 week tot betere resultaten leidt. Zowel de SCORAD (scoring atopic dermatitis) als de DLQI (dermatology life quality index, een gevalideerde kwaliteit van leven score) verbeterden significant gedurende zowel de 3 weken als de 4 weken durende kuur. Er kon geen verschil worden aangetoond tussen de beide groepen. Wel waren de 4 weken behandelde patiënten, in tegenstelling tot de 3 weken follow-up.

In **hoofdstuk 3** wordt de succesvolle UVA-1 therapie van patiënten met dyshidrotisch eczeem beschreven. Het effect van UVA-1 therapie werd onderzocht in een dubbel blinde, placebo gecontroleerde studie. UVA-1 therapie bleek significant beter dan placebo behandeling,

waarmee dyshidrotisch eczeem kon worden toegevoegd aan de T-cel gemedieerde indicaties voor UVA-1 lichttherapie.

Lichen ruber planus, eveneens een T-cel gemedieerde aandoening, is meestal slechts op de predilectieplaatsen gelocaliseerd en wordt dan locaal behandeld. Wanneer er sprake is van een gegeneraliseerde vorm is locale therapie niet praktisch. In **hoofdstuk 4** beschreven wij het positieve effect van UVA-1 therapie bij patiënten met chronische, gegeneraliseerde lichen ruber planus. In dit hoofdstuk wordt bovendien het toxische effect van UVA-1 monotherapie op het lymfocytaire infiltraat gedemonstreerd. UVA-1 lichttherapie kan bij deze uitgebreide vorm een goed alternatief zijn.

Aangezien UVA-1 effectief is gebleken in de behandeling van dermale T cel gemedieerde aandoeningen, kan men verwachten dat UVA-1 ook in staat zou kunnen zijn de dermale capillairen te bereiken en de daarin circulerende cellen te beïnvloeden. Behalve T cellen zouden ook B cellen beïnvloed kunnen worden. Systemische lupus erythematodes (SLE) wordt gekenmerkt door de productie van auto-antilichamen door geactiveerde B-lymfocyten, wat aanleiding geeft tot inflammatie in diverse organen. De gebruikelijke therapeutische mogelijkheden, zoals systemische corticosteroïden, azathioprine en cyclofosfamide kunnen gepaard gaan met verscheidene, mogelijk ernstige bijwerkingen.

Vanwege het bekende risico op fotosensitiviteit onderzochten wij in eerste instantie het effect van slechts 6 J/cm² UVA-1, in een dubbel blinde, placebo gecontroleerde, cross-over studie (hoofdstuk 5). Deze behandeling resulteerde na 3 weken in een verlaging van zowel de SLE activity measure (SLAM) als de SLE disease acitivity index (SLEDAI), twee gevalideerde methodes om ziekteactiviteit van SLE patiënten te meten. Het verschil met het effect van placebo behandeling was echter niet statistisch significant. Met de vraag of een hogere dosis een beter effect heeft werden in een tweede studie volgens de zelfde opzet SLE patiënten behandeld met 12 J/cm² (hoofdstuk 6). Na 3 weken behandeling met 12 J/cm² UVA-1 werd een significante verbetering gezien van zowel de SLAM als de SLEDAI. Bovendien bleek UVA-1 behandeling significant beter dan placebo behandeling, gemeten volgens de SLAM. In beide *in vivo* studies werd een effect op auto-antilichamen titers waargenomen. Behoudens problemen van passagère fotosensitiviteit bij enkelen en geringe activatie van subacute cutane lupus erythematosus (SCLE) bij een patiënt, traden tijdens beide studies geen bijwerkingen op.

In deze gecontroleerde studies kon bevestigd worden dat UVA-1 een gunstig effect kan hebben in de behandeling van matig actieve SLE (hoofdstuk 5 en 6). Het auto-antilichamen profiel van de patiënten was erg heterogeen, zoals vaak het geval is bij SLE patiënten. Desondanks werd een duidelijke negatieve trend gezien in de anti-SSA titers na UVA-1 behandeling in de eerste studie.

Deze waarneming en de afname van de anti-RNP titer en de anti-SSA titer van twee patiënten in de tweede studie, brachten ons ertoe in een *in vitro* studie het effect van UVA-1 op de immunoglobuline productie door B cellen te onderzoeken (hoofdstuk 7). Toxisch effect op de geactiveerde B-lymfocyten en plasmacellen in de bloedcapillairen van de huid zou kunnen resulteren in afname van immunoglobuline productie met als mogelijk gevolg vermindering van de ziekteactiviteit van SLE patiënten.

Ten eerste werd de penetratie van UVA-1 door de epidermis bepaald. Ongeveer 40% van het UVA-1 bleek de dermis te kunnen bereiken. Vervolgens was het belangrijk te weten hoe gevoelig B-lymfocyten zijn voor het cytotoxische effect van UVA-1. Vierentwintig uur na belichting met UVA-1 bleek bij oplopende doses UVA-1 een toenemend aantal perifere bloed mononucleaire cellen (PBMCs) dood te gaan. Na pre-incubatie van de celkweken met catalase, hetgeen waterstofperoxide afbreekt, werd celdood volledig voorkomen. Bij onderzoek naar de toxiciteit van UVA-1 voor verschillende subpopulaties van de PBMCs, bleek de monocyten populatie de meest gevoelige voor UVA-1 cytotoxiciteit.

Tenslotte werd naar het effect van UVA-1 op de immunoglobuline productie door geactiveerde B-lymfocyten van gezonde proefpersonen gekeken. De IgM-, IgG- en de IgE productie nam af bij oplopende doses UVA-1, in de IL-10 of IL-4 gestimuleerde condities. Een vergelijkbaar, doch niet statistisch significant, effect was waarneembaar op de IgA productie.

Hoewel in onze *in vitro* studie 20% van de PBMCs dood ging na 6 J/cm² UVA-1, was dit te weinig om de waargenomen afname van IgM en IgE productie onder invloed van deze zelfde dosis volledig te verklaren. Behalve celdood speelt mogelijk ook UVA-1 geïnduceerde afname van B-cel functie een rol. Waarschijnlijk speelt de productie van waterstofperoxide een belangrijke rol in het tot stand komen van deze toxische effecten van UVA-1 straling.

Samenvattend kan UVA-1 behalve bij de behandeling van T cel gemedieerde aandoeningen ook effectief zijn in de behandeling van een B cel gemedieerde ziekte als systemische lupus erythematodes. Het zou interessant zijn de effectiviteit van UVA-1 lichttherapie bij andere auto-immuunziekten systematisch te onderzoeken. Een voorwaarde is echter dat bij deze aandoeningen geactiveerde B cellen/plasmacellen in de dermale capillairen aanwezig zullen zijn.

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Curriculum vitae

Marloes C.A. Polderman werd geboren op 29 september 1971 te Leiden. Na het behalen van haar VWO-B diploma in 1989 aan het Sint Thomascollege te Venlo, begon zij in september 1989 aan haar studie geneeskunde aan de Rijksuniversiteit Leiden. Zij haalde in 1990 haar propedeuse examen, in augustus 1994 haar doctoraalexamen en in december 1996 haar artsexamen. In januari 1997 begon zij als assistent geneeskundige niet in opleiding (AGNIO) aan de afdeling Dermatologie van het Leids Universitair Medisch Centrum (LUMC) aan een onderzoek naar het effect van de Ruby laser als ontharingstherapie bij patiënten met overbeharing. Ook deed zij onderzoek naar het effect van UVA-1 koudlichtbehandeling bij patiënten met constitutioneel eczeem. Na een subsidie van Het Nationaal Reumafonds voor onderzoek naar het effect van UVA-1 bij patiënten met systemische lupus erythematosus, kreeg zij in 1999 een aanstelling als assistent geneeskundige in opleiding tot klinisch onderzoeker (AGIKO) aan de afdeling Dermatologie van het LUMC. Van juni 2000 tot mei 2005 was zij in opleiding tot dermatoloog. In deze periode werd eveneens het promotieonderzoek afgerond. Sinds 1 juni 2005 is zij als dermatoloog werkzaam in het Flebologisch Centrum Oosterwal in Alkmaar en in het Haga Ziekenhuis in Den Haag (locatie Levenburg).

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