16

PREVENTION OF POLYMORPHOUS LIGHT ERUPTION BY A NEW BROADSPECTRUM SUNSCREEN: NEED FOR A HIGH UVA PROTECTING FACTOR.

D. Moyal[1], O. Binet[2], A. Richard[3], A. Rougier[3], C. Hourseau[1]
[1]L'Oréal Resarch, Clichy, France. [2]Department of Dermatology, Rothschild Fundation, Paris, France. [3]La Roche-Posay Pharmaceutical Laboratories, Courbevoie, France.

Polymorphous light eruption (PLE) represents an abnormal response of human skin to UV radiations characterized by interindividual polymorphic but intraindividual monomorphic skin lesions. It is now well established that UVA radiations (320-40 nm) are predominant in the elicitation of this skin disease. Commonly used strategies to protect human skin against deleterious effects exerted by UV include topical application of sunscreen formulations.

The aim of the present work was to assess, in human volunteers under real sun exposure conditions, the efficacy of a new broadspectrum sunscreen product in the prevention of PLE. This sunscreen has both a very high sun (SPF>60) and UVA (UVAPF=28, Persistent Pigment Darkening method (PPD)) protection factors. Morover, the following UV filtering system: UVB filter (Octocrylene), UVA filters (Mexoryl SX, Mexoryl XL, Parsol 1789), TiO^2 allows the formula to be photostable.

The efficacy of this new broaspectrum sunscreen was compared to that obtained with a classical sunscreen having a similar SPF but a lower protection factor in the UVA (UVA-PF=3). This comparative study was performed by half body on the same patients. 16 female volunteers susceptible to PLE were progressively sun exposed during 6 days. They received a total UV dose equivalent to 50 MED and 300 J/cm^2 of UVA. Under these intensive sunlight exposures, PLE was induced in 15 patients treated with the sunscreen formula having a low UVA-PF value, whereas only 4 cases of delayed PLE were recorded when using the new broadpectrum sunscreen.

This study clearly demonstrate that two sunscreen formulations having similar SPF values are not equivalent in preventing from PLE and that there is a need for products covering the entire UV spectrum, i.e., UVB + UVA.

PREVENTION OF POLYMORPHOUS LIGHT ERUPTION BY A NEW BROADSPECTRUM SUNSCREEN: NEED FOR A HIGH UVA PROTECTING FACTOR

D. Moyal [1], O. Binet [2], A. Richard [3], A. Rougier [3], C. Hourseau [1]

[1] L'ORÉAL Research, Clichy, FRANCE. [2] Fondation Rothschild, Paris, FRANCE. [3] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE.

INTRODUCTION

UVA play an essential role in triggering sunlight eruptions, even if these conditions are triggered by radiations spanning the whole spectrum of ultraviolet light. Consequently, researching the best UVA-blocking system seemed essential for protecting sensitive subjects, and particularly those subjects who develop polymorphous light eruption (PLE) from their first exposures to sunlight.

A new filter, Mexoryl®XL, was added to the existing high-performance combination of UVA filters Mexoryl®SX and Avobenzone (Parsol® 1789)(1), which allows a yet unequalled level of protection against UVA to be obtained (SPF-UVA = 28).

The efficacy of this new formulation in preventing the occurrence of PLE was studied following exposure of one half of the body to the sun, in comparison with a commercial formulation having a similar solar protection factor (SPF > 60), but with a lower UVA filtering power.

MATERIAL AND METHODS

PRODUCTS

CREAM A SPF > 60 * UVA-PF 28 **	*UVB Filter* : Octocrylene *UVA Filters* : Terephthalylidene dicamphor sulfonic acid (Mexoryl®SX) Drometrizole Trisiloxane (Mexoryl®XL) Avobenzone (Parsol®1789) TiO_2
CREAM B SPF > 60 UVA-PF 3.5 **	*UVB Filters* : Isoamyl p-methoxycinnamate Octyl triazone 4-Methylbenzylidene camphor *UVA Filter* : Avobenzone TiO_2

* SPF determined according to the FDA method (2).
** UVA-PF determined according to the persistent pigment darkening (PPD) method (3).

SUBJECTS

16 women with a predisposition for PLE, with no circulating antinuclear factors, categorized as phototype II or III, aged 22 to 51 years. The volunteers were especially sensitive to PLE and had had no exposure to sunlight during at least 3 months.

APPLICATION OF THE PRODUCTS

The products were applied on one half of the body by the subjects themselves, before and during each new exposure. The allocation of the product to a given half of the body was randomized.

EXPOSURES

The volunteers were exposed to the sunlight during 6 days, once in the morning and once in the afternoon, at increased doses of UV. The UVA and total UV erythematous doses were recorded with a PMA radiometer from Solar-Light.

Days	D1 → D6		Total
UVA Doses Joules/cm²	40 J/cm²	60 J/cm²	300 J/cm²
MED *	7 MED	12 MED	52 MED
Duration	3 hours	5 hours	24 hours

* Minimal Erythema Dose

The UVA doses to be given were selected based on previous studies, in order to be realistic.

The doses of UV received did not cause erythema due to the products' very high protecting power (SPF).

CLINICAL EVALUATIONS

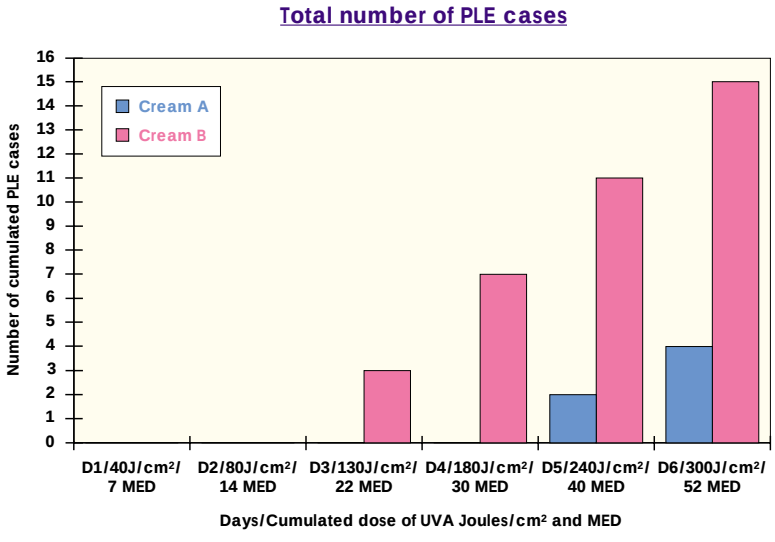
The assessments were made each evening by the dermatologist, who was blinded to the nature and allocation of the products in each volunteer. The signs characteristic of PLE were scored: reticular erythema, papulae, pruritus. The test area was the upper part of the chest (constant area of occurrence of PLE), the other areas being used to confirm the first result.

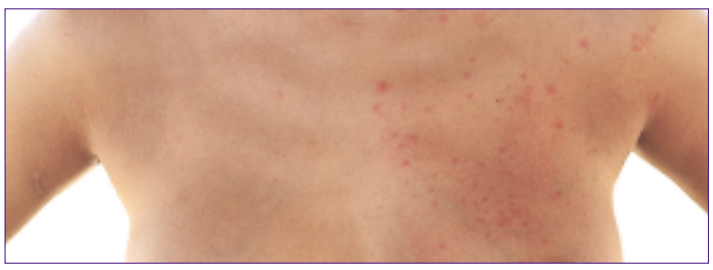
RESULTS

With Cream B, 15 subjects out of 16 developped PLE between Day 3 and Day 6. Conversely, only 4 of these subjects developped PLE with Cream A. Moreover, the occurrence of PLE was delayed compared to the half-chest treated with Cream B.

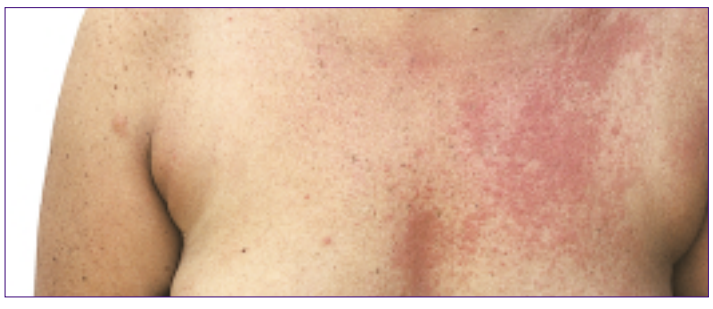
Cream B was not efficacious enough to prevent PLE (94% of occurrences). On the other hand, Cream A markedly prevented PLE (75% of cases), or delayed or decreased its severity (25% of cases).

Total number of PLE cases





Cream A — Cream B



Cream A — Cream B

Examples of obtained reactions

CONCLUSION

The present study confirmed the role of UVA in triggering PLE as well as the need for highly protective products covering the whole spectrum of UVB and UVA.

Two products with similar SPFs were not equivalent in terms of prevention of PLE.

The product with a high protection factor against UVA, as determined by the persistent pigment darkening method (PPD) was markedly superior to the product with a lower protection factor in this domain of the UV spectrum. This underlines the specific advantage and superiority of the product with a higher protection power in the UVA domain. The level of protection evaluated according to the PPD method seems associated to a marked efficacy in the prevention of PLE.

REFERENCES

1. Moyal D, Binet O: Polymorphous Light Eruption (PLE): its reproduction and prevention by sunscreens. In 'Sunscreens: Development, Evaluation and Regulatory Aspects', 2nd edition (N Lowe, N Shaath, M Pathak, ed.; Marcel Dekker Inc). pp 611-617. 1996.
2. U.S. Food and Drug Administration (FDA). Sunscreen drug products for over-the-counter human use; Tentative final monograph; proposed rule. *Federal Register, 58:* 28194-28302, 1993.
3. Japan Cosmetic Industry Association (J.C.I.A): Measurement standards for UVA protection efficacy. Nov. 21, 1995.

LA ROCHE-POSAY LABORATOIRE PHARMACEUTIQUE

L'ORÉAL RECHERCHE

SUNSCREENS WITH HIGH SPF VALUES ARE NOT EQUIVALENT IN THE PROTECTION FROM UVA INDUCED POLYMORPHOUS LIGHT ERUPTION

H. STEGE [1], M. BUDDE [1], S. GRETHER-BECK [1], A. RICHARD [2], A. ROUGIER [2], T. RUZICKA [1], J. KRUTMANN [1]

[1] *Clinical and Experimental Photodermatology, Department of Dermatology, University of Düsseldorf, GERMANY* [2] *- La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE*

INTRODUCTION

Polymorphous light eruption (PLE) is the most frequent photodermatosis with an estimated incidence of approximately 3-17% (1). Photoprovocation testing has revealed that the vast majority of PLE patients (> 80%) is sensitive to longwave ultraviolet (UV) radiation, that is radiation in the range of 340-400 nm (UVA1) (2). Strategies directed at prevention of PLE therefore include sunscreens which preferentially absorb in the UVA range.

The efficacy of sunscreens is usually indicated by their protection factors (3). Accordingly, the sun protection factor (SPF) reflects the capacity of a given sunscreen to prevent solar radiation-induced erythema, and the pigment darkening factor serves as a measure for the UVA protective capacity. Measurement of the SPF follows clear rules that have been defined and standardized by the COLIPA. In contrast, the pigment darkening factor may be determined by two different methods, that is the immediate pigment darkening method and the persistant pigment darkening method. The relationship between these factors and the capacity of a given UVA-absorbing sunscreen to protect PLE patients from developing skin lesions is currently unknown. Here we have therefore compared three different and commercially available sunscreens with defined SPFs for their capacity to prevent the development of skin lesions in PLE patients undergoing photoprovocation testing. The sunscreens that we have compared were characterized by very high SPFs and thus did not differ significantly in their capacity to reduce the amount of UVB radiation penetrating into the skin (between 2 and 4%; table 1). Two of them were also known to have a high UVA-PF, reducing the amount of UVA radiation penetrating into human skin by 94% - 96%.

MATERIAL AND METHOD

Patients:

Thirteen patients (6 female, 7 male) with a history of PLE and positive photoprovocation testing were enrolled after written informed consent was obtained.

Photoprovocation testing:

In order to provoke the development of skin lesions in PLE patients, individual predilection sites (forearms and back) were exposed on three consecutive days to 100 J/cm^2 UVA radiation from a Sellamed 2000 irradiation device (Sellas Systems) at an intensity of 60 mW/sec. Irradiated skin sites were evaluated for the development of skin lesions immediately and 24 hours after each exposure.

Sunscreens:

Three different sunscreens were assessed. Details including the type of absorber present in each sunscreen, SPF, UVA-PF, and the capacity of each sunscreen to reduce the amount of UVB or UVA radiation penetrating into skin are given in table 1. Each sunscreen was applied 20 minutes prior to irradiation on a given test area of 6 x 6 cm according to the COLIPA norm.

RNA Extraction and RT-PCR Analysis. In 3 of 13 patients, 4 mm punch biopsy specimens were obtained from unirradiated control skin, from an unprotected and from a sunscreen C-pretreated photoprovocation test site 24 hours after the last irradiation. Intercellular adhesion molecule-1 mRNA expression was assessed in a semiquantitative manner by differential RT-PCR. This method has previously been used for analysis of in-situ expression of specific mRNAs and found to be highly sensitive and reliable (4-6). Each PCR of each sample was carried out at least two times. Products were visualized by gel analysis using ethidium bromide staining.

Table 1:
Description of the tested sunscreens

Sunscreen	UVB Absorber	SPF (UVB)	Reduction of UVB in %	UVA Absorber	UVA-PF	Reduction of UVA in %
A	Eusolex® 6300 Parsol® MCX Uvinul® T 150 Neoheliopan	> 75	> 98.6	Parsol® 1789 TiO_2	15 (IPD method)*	93.3
B	Eusolex® 6300 Parsol® MCX Uvinul® T 150	35	97.1	Parsol® 1789 TiO_2	unknown	unknown
C	Octocrylene	> 60	> 98.3	Mexoryl® SX Mexoryl® XL Parsol® 1789 TiO_2	28 (PPD method)**	96.43

* **Immediate Pigment Darkening method (IPD method)**
** **Persistent Pigment Darkening method (PPD method)**

RESULTS

Photoprovocation testing was positive in all patients assessed. The capacity of the three sunscreens tested to provide protection against the development of skin lesions in these patients markedly differed (Photo 1). Sunscreen A provided protection in 6 out of 13 patients, sunscreen B in 3 out of 13 patients, and sunscreen C in 13 out of 13 patients.

Development of skin lesions in irradiated skin areas was associated with an increased expression of keratinocyte ICAM-1 mRNA expression (Photo 2). Application of sunscreen C to test areas prior to photoprovocation testing completely prevented not only the development of skin lesions, but also the increase in ICAM-1 mRNA expression (Photo 2).

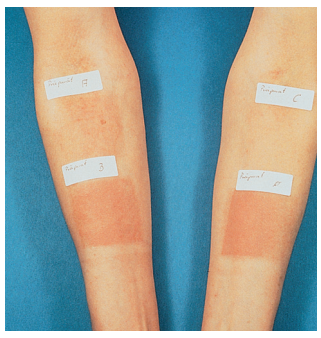


Photo 1:
Photoprovocation test reactions 24 hours after the 3rd UVA radiation exposure in a patient with polymorphous light eruption. Prior to irradiation, test sites were either left unprotected (left arm, lower test site) or protected with suncreens A, B or C as indicated.

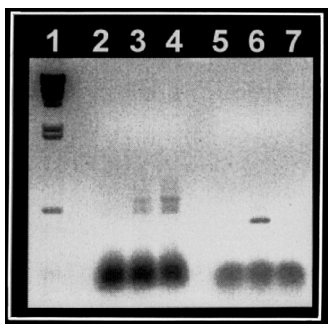


Photo 2:
Semiquantitative RT-PCR for ICAM-1 mRNA expression in photoprovocation test areas 24 hours after the 3rd UVA radiation exposure of a PLE patient. Biopsies were obtained from unirradiated control skin (no UVA), unprotected, UVA-irradiated skin (UVA) or sunscreen C-pretreated, UVA-irradiated skin. ICAM-1 mRNA expression was assessed by semiquantitative RT-PCR as described in Material and Method and visualized by gel analysis. Lane 1: lambda HindIII standard; lane 2: b-actin mRNA expression in unirradiated control skin; lane 3: b-actin mRNA expression in unprotected, UVA-irradiated skin; lane 4: b-actin mRNA expression in sunscreen C-pretreated, UVA-irradiated skin; lane 5: ICAM-1 mRNA expression in unirradiated control skin; lane 6: ICAM-1 mRNA expression in unprotected, UVA-irradiated skin; lane 7: ICAM-1 mRNA expression in sunscreen C-pretreated, UVA-irradiated skin.

DISCUSSION

In the present study we have compared the capacity of three different sunscreens to protect PLE patients by employing a photoprovocation protocol, in which predilection sites are exposed for 3 consecutive days to daily exposures with 100 J/cm^2 of UVA radiation. All patients tested had a history of positive photoprovocation testing using this standard protocol. We have selected UVA-sensitive PLE patients, because they represent the vast majority of cases and may thus be regarded as prototypic for this particular photodermatoses (2). By employing the above mentioned standard photoprovocation protocol it was observed that positive photoprovocation results could be obtained in 100% of tested patients. This observation indicates that the photoprovocation protocol used is characterized by a high intraindividual reproducibility. It is therefore ideally suited to evaluate measures directed at the prevention of UVA radiation-induced skin lesions in PLE patients.

Among these measures, sunscreens have previously been reported to be of benefit for PLE patients (1). This is in agreement with the present observation that topical application of sunscreens prior to photoprovocation testing prevented the development of skin lesions in PLE patients. The capacity of the 3 sunscreens tested in the present study to provide protection, however, varied markedly. This finding was somewhat surprising, since the tested sunscreens did not differ significantly when compared for their ability to reduce the amount of UVB or UVA radiation penetrating into the skin, as indicated by their high SPF and UVA-PF values. The major difference between the 3 sunscreens is given by the type of UV-filtering systems present in each sunscreen. It appears that Mexoryl® SX plus Mexoryl® XL is the most efficient combination providing 100% protection.

Effective prevention of clinically apparent skin lesions in PLE patients through application of sunscreen C was associated with complete inhibition of UVA radiation-induced expression of ICAM-1 mRNA expression in human keratinocytes. This observation further supports the concept that PLE represents an abnormal response of human skin towards UVA radiation that differs at a quantitative level by showing an overshooting and sustained expression of proinflammatory molecules such as ICAM-1 (7). Recent *in vitro* studies indicate that UVA radiation-induced keratinocyte ICAM-1 expression is mediated through the generation of singlet oxygen (8,9). It is therefore tempting to speculate that sunscreen C, that contains the combination of Mexoryl®XL and Mexoryl®SX, is particularly well suited to protect human skin from UVA radiation-induced generation of singlet oxygen. The combination of these two UV-filtering systems may thus not only be of benefit for protection of PLE patients, but may also be superior to conventional sunscreens in protecting against other UVA radiation-induced, singlet oxygen-mediated biological effects. These could possibly include protection against singlet oxygen-mediated upregulation of matrix metalloproteinase I, II and III (10) expression in human dermal fibroblasts or the UVA radiation-induced generation of large scale deletions in mitochondrial DNA in dermal fibroblasts (11,12). Both effects are thought to be pivotal to UVA radiation-induced actinic damage in human skin, thus making sunscreen C a prime candidate for prevention of photoaging.

REFERENCES

1. Hönigsmann H. Polymorphous light eruption. In: Lim HW, Soter NA, eds. *Clinical Photomedicine*. New York: Marcel Dekker, 167-180 (1993)
2. Hölzle E, Plewig G, Hofmann C, Roser-Maass E. Polymorphous light eruption. Experimental reproduction of skin lesions. *J Am Acad Dermatol*, **7**: 111-125 (1982)
3. Lowe NJ, Shaath NA, Pathak MA, eds. Sunscreens. 2nd ed. New York: Marcel Dekker: (1997)
4. Henninger HP, Hoffmann R, Grewe M, Schulze-Specking A, Decker K. Purification and quantitative analysis of nucleic acids by anion-exchange-high-performance liquid chromatography. *Biol Chem Hoppe-Seyler,* **374**:625-629 (1993)
5. Grewe M, Gyufko K, Scköpf E, Krutmann J. Lesional expression of interferon-γ in atopic eczema. *Lancet,* **343**: 23-25 (1994)
6. Ahrens C, Grewe M, Berneburg M, Grether-Beck S, Quilliet X, Mezzina M, Sarasin A, Lehmann AR, Arlett CF, Krutmann J. Photocarcinogenesis and inhibition of intercellular adhesion molecule 1 expression in cells of DNA-repair-defective individuals. *Proc Natl Acad Sci USA* **94**: 6837-6841 (1997)
7. Norris PG. Advances in understanding the pathogenesis of photodermatoses. *Curr Op Dermatol*, **1**: 185-190 (1993)
8. Grether-Beck S, Olaizola-Horn S, Schmitt H, Grewe M, Jahncke A, Johnson JP, Briviba K, Sies H, Krutmann J. Activation of transcription factor AP-2 mediates UVA radiation- and singlet oxygen-induced expression of the human intercellular adhesion molecule-1 gene. *Proc Natl Acad Sci USA,* **93**:14586-14591 (1996)
9. Grether-Beck S, Krutmann J. Ultraviolet A radiation-induced expression of human genes: Molecular and photobiological mechanisms. *Biol Chem,* **378**:1231-1236 (1997)
10. Wlaschek M, Briviba K, Stricllin GP, Sies H, Scharfetter-Kochanek K. Singlet oxygen may mediate the ultraviolet A-induced synthesis of interstitial collagenase. *J Invest Dermatol,* **104**:194-198 (1995)
11. Berneburg M, Gattermann N, Stege H, Grewe M, Vogelsang K, Ruzicka T, Krutmann J. Chronically ultra violet-exposed human skin shows a higher mutation frequency of mitochondrial DNA as compared to unexposed skin and the hematopoietic system. *Photochem Photobiol,* **66**:271-275 (1997)
12. Berneburg M, Kürten V, Grether-Beck S, Ruzicka T, Klotz LO, Sies H, Krutmann J. Induction of mito chondrial (MT) DNA mutations in human fibroblasts by *in vitro* ultraviolet A (UVA) irradiation. *J Invest Dermatol* , **110**: 489A (1998)

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

18

PREVENTION OF SOLAR INDUCED IMMUNOSUPPRESSION BY A NEW HIGHLY PROTECTIVE BROADSPECTRUM SUNSCREEN.

D. Moyal[1], L. Duteil[2], C. Queille-Roussel[2], J.P. Ortonne[2], C. Hourseau[1], A. Rougier[3]
[1]L'Oréal Resarch, Clichy, France. [2]CPCAD, Nice, France. [3]La Roche-Posay Pharmaceutical Laboratories, Courbevoie, France.

It is now well established that ultraviolet radiations are responsible for alterations of the cutaneous immune system and may be at the ethiology of skin cancers. Recently, it has been clearly demonstrated that not only UVB (290-320 nm) but also UVA (320-400 nm) can be responsible of these effects. Thus, sunscreen products highly protective in the UVB range (erythema) are less effective in preventing the UV induced changes in the skin immune function than those covering the entire UVB + UVA spectrum.

We previously studied in human the effect of exposure to either UVB + UVA or only UVA on the delayed-type hypersensitivity response (DTH). DTH was assessed using a Multitest kit (Pasteur/Mérieux), providing an original approach to evaluate modifications in the cutaneous immune capacities.

The aim of the present work was to evaluate, in humans volunteers, under real sun exposure conditions, the efficacy of a new broadspectrum sunscreen product in preventing loss of DTH response.

DTH tests were performed before and after sun exposure of the upper part of the back. A non exposed area (forearm) was used as control. Prior to sun exposures 14 subjects of were treated with the new sunscreen formula. This sunscreen has both a very high sun (SPF>60) and UVA (UVAPF=28, Persistent Pigment Darkening method (PPD)) protection factors. Morover, the following UV filtering system: UVB filter (Octocrylene), UVA filters (Mexoryl SX, Mexoryl XL, Parsol 1789), TiO^2 allows the formula to be photostable.

The volunteers were sun exposed during 6 days. They received a total UV dose equivalent to 64 MED and 400 J/cm^2 of UVA. Compared to the DTH response we obtained before sun exposure, we did not detect any changes in the immune response when skin was protected by the sunscreen formula.

We have demonstrated that, under intensive sunlight exposure, the use of a highly protective UVB + UVA sunscreen can prevent from photo-immunosuppression. This is of particular importance if we consider the possible link between immunosuppression and skin cancers developments.

PREVENTION OF SOLAR INDUCED IMMUNOSUPPRESSION BY A NEW HIGHLY PROTECTIVE BROADSPECTRUM SUNSCREEN

D. MOYAL [1], L. DUTEIL [2], C. QUEILLE-ROUSSEL [2], JP. ORTONNE [2], C. HOURSEAU [1], A. RICHARD [3], A. ROUGIER [3]

[1] L'ORÉAL Recherche, Clichy, FRANCE. - [2] CPCAD, Nice, FRANCE - [3] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE

INTRODUCTION

It is now well established that ultraviolet radiations are responsible for alterations of the cutaneous immune system and may be at the ethiology of skin cancers. Recently, it has been clearly demonstrated that not only UVB (290-320 nm) but also UVA (320-400 nm) can be responsible of these effects. Thus, sunscreen products highly protective in the UVB range are less effective in preventing the UV induced changes in the skin immune function than those covering the entire UVB + UVA spectrum (1-6).
We previously studied in human the effect of exposure to either UVB + UVA or only UVA on the delayed-type hypersensitivity response (DTH).(3-4) DTH was assessed using a Multitest kit (Pasteur/Mérieux), providing an original approach to evaluate modifications in the cutaneous immune capacities (1,3,4).
A previous study in non protected volunteers (n=10), on a period of 12 days sun exposure (12 MED, 60 J/cm^2) showed a highly significant decrease (- 35%) in skin reaction to the Multitest.
The aim of the present work was to evaluate, in human volunteers, under real sun exposure conditions, the efficacy of a new broadspectrum sunscreen product in preventing loss of DTH response.

MATERIAL AND METHOD

Delayed type hypersensitivity test: Multitest Pasteur/Merieux Kits

ANTIGENS	
1 - Tetanus	5 - Negative control a 70 % sterile glycerin solution
2 - Diphteria	6 - Candida albicans
3 - Streptococcus (group C)	7 - Trichophyton mentagrophytes
4 - Tuberculin	8 - Proteus mirabilis

Measurements of DTH responses were performed 48 hours after application of the Multitest. The diameter of each positive response, identified as local induration, was measured in two directions. These two diameters were then averaged. The total score was calculated by adding the individual score corresponding to each antigen.

Subjects studied

14 volunteers participated to the study.
Inclusion criteria comprised skin type II, III or IV, age between 18 and 50, in general good health and having an initial DTH response to the Multitest superior to 8 (total score).
Exclusion criteria were medications causing immunomodulation or risk of photosensitization.

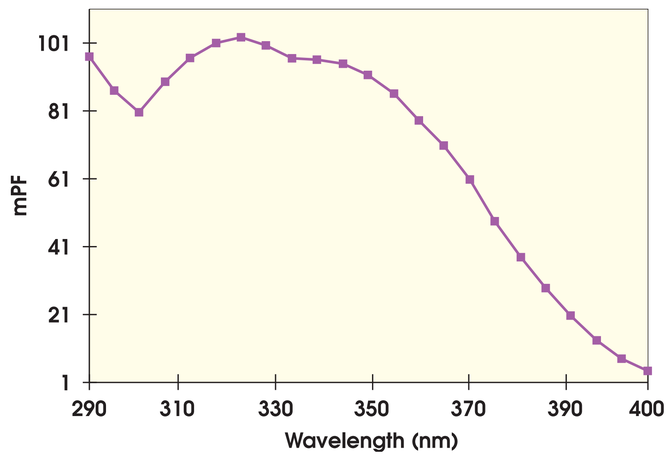
Product

- *UVB filter : Octocrylene*
- *UVA filters : Terephthalylidene dicamphor sulfonic acid (Mexoryl®SX)* Drometrizole Trisiloxane (Mexoryl®XL) Avobenzone (Parsol®1789)*
- *TiO_2*

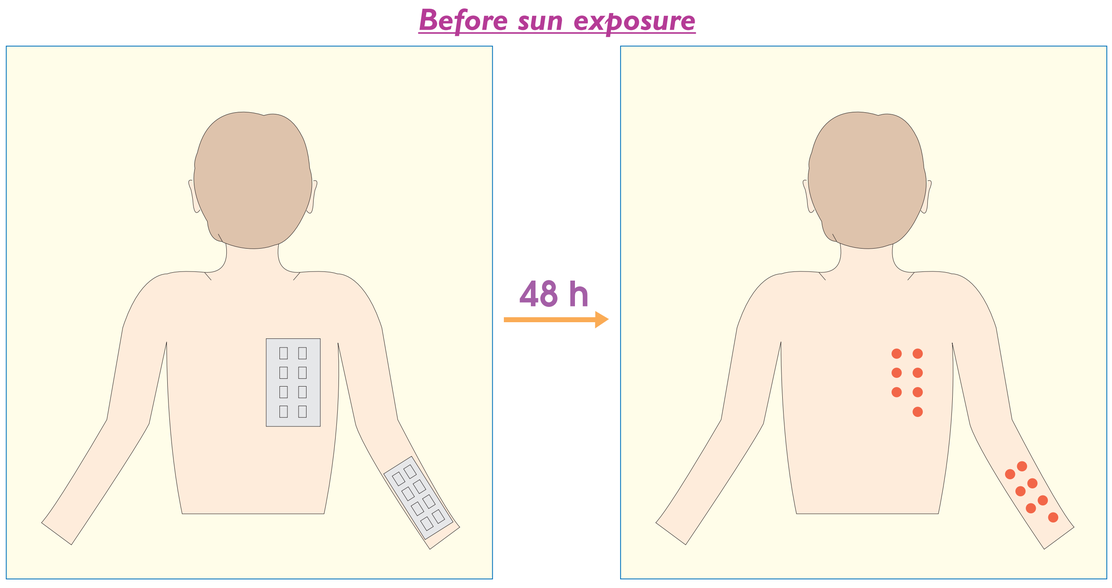
* USAN Name: ecamsule

This sunscreen has both a very high Sun Protection Factor (SPF60+) (7) and a very high UVA protection factor UVA-PF 28 assessed by the Persistent Pigment Darkening (PPD) method.(8)

Monochromatic Protection Factor curve



Before sun exposure

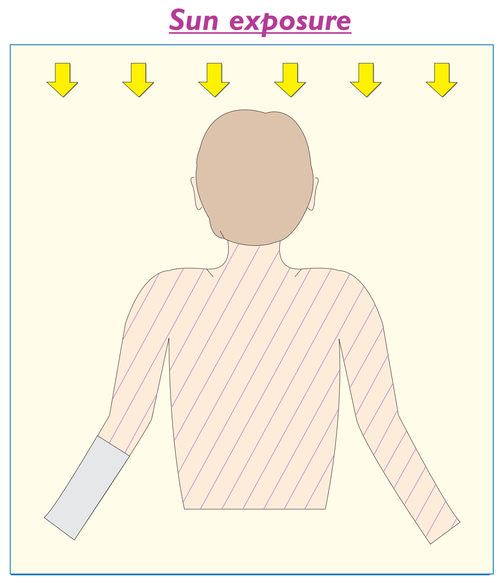


Application of the Multitests — **Measurements of the DTH responses**

during 6 days
UV doses progressively increased
8 MED → 13.3 MED
53 J/cm2 UVA → 80 J/cm^2 UVA
3 hours → 5 hours
total dose received: 64 MED and 400 J/cm^2 of UVA

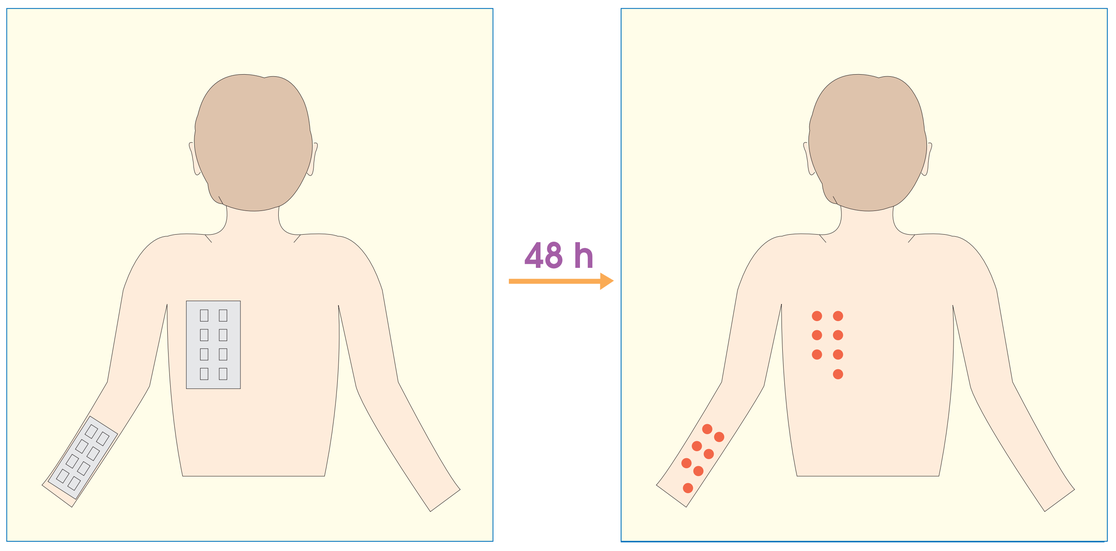
The broadspectrum sunscreen was applied (between 0.5 and 1 mg/cm^2) before and during each exposure on the whole body excepted the forearm which was protected by a armband impervious to UV rays.

Sun exposure



Armband — Broadspectrum sunscreen

48 hours after sun exposure



Application of the Multitests — **Measurements of the DTH responses**

C O M P A R I S O N

RESULTS

Efficacy of the sunscreen DTH test responses : Comparison of total score mean ± SD (± sem)

TEST SITE	*Pre-UV* exposed site (back)	*Post-UV* exposed site (back)	*Pre-UV* unexposed site (forearm)	*Post-UV* unexposed site (forearm)
TOTAL SCORE	14.6 ± 5.2 (± 1.4)	11.3* ± 5.3 (± 1.4)	7.3 ± 3.7 (± 1)	5.9* ± 3.8 (± 1)

* not significantly different from pre-UV for each site ($p > 0.05$)

Compared to the DTH response we obtained before sun exposure, we did not detect any changes in the immune response when skin was protected by the sunscreen formula.

CONCLUSION

Under intensive sunlight exposure and realistic application conditions, the use of a highly protective UVB + UVA sunscreen can prevent from the photoinduced-immunosuppression.
This is of particular importance if we consider the possible link between immunosuppression and skin cancer developments.

REFERENCES

1. Hersey P, MacDonald M, Burns C, Schibeci S, Matthews H, Wilkinson FJ: Analysis of the effect of a sunscreen agent on the suppression of natural killer activity induced in human subjects by radiation from solarium lamps. *J Invest Dermatol.* **88** : 271-276 (1987)
2. Bestak R, Barnetson SR, Near M, Halliday GM: Sunscreen protection of contact hypersensitivity responses from chronic solar-simulated ultraviolet irradiation correlates with the absorption spectrum of the sunscreen. *J Invest Dermatol.* **105**: 345-351 (1995)
3. Moyal D, Courbière C, Le Corre Y, de Lacharrière O, Hourseau C : Immunosuppression induced by chronic solar-simulated irradiation in humans and its prevention by sunscreens. *Eur J Dermatol.* **7** : 223-225 (1997)
4. Moyal D: Immunosuppression induced by chronic ultraviolet irradiation in humans and its prevention by sunscreens. *Eur J Dermatol.* **8** : 209-211 (1998)
5. Damian DL, Halliday GM, Barnetson RSC: Broadspectrum sunscreens provide greater protection against ultraviolet-radiation-induced suppression of contact hypersensitivity to a recall antigen in humans. *J Invest Dermatol.* **109** : 146-151 (1997)
6. Serre I, Cano JP, Picot MC, Meynadier J, Meunier L: Immunosuppression induced by acute solar-simulated ultraviolet exposure in humans: Prevention by a sunscreen with a sun protection factor of 15 and high UVA protection. *J Am Acad Dermatol.* **37** : 187-194, (1997)
7. U.S. Food and Drug Administration (FDA). Sunscreen drug products for over-the-counter human use ; Tentative final monograph ; proposed rule. *Federal Register*, **58** : 28194-28302 (1993)
8. Japan Cosmetic Industry Association (J.C.I.A): *Measurement standards for UVA protection efficacy*. Nov. 21 (1995)

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

19

UV-INDUCED PRODUCTION OF IMMUNOSUPPRESSIVE MEDIATORS IN HUMAN SKIN: PREVENTION BY A BROADSPECTRUM SUNSCREEN.

H. Kalden[1], T. Brzoska[1], T. Schwarz[1], A. Richard[2], A. Rougier[2], T. Luger[1]
Ludwig Boltzmann Institute of Cell Biology and Immunology of the Skin, Dept. of Dermatology, University of Münster, Germany. La Roche-Posay Pharmaceutical Laboratories, La Roche-Posay and Courbevoie, France.

Irradiation of the skin with UV-light is well known to cause local as well as systemic immunosuppression. Therefore, the protective effect of a new broad spectrum sunscreen on the UV-induced production of immunosuppressive mediators interleukin-10 (IL-10) and α -melanocyte stimulating hormone (αMSH) was investigated.
The broad spectrum sunscreen used has both a very high sun (SPF>60) and UVA (UVAPF=28, Persistent Pigment Darkening method (PPD)) protection factors. Morover, the following UV filtering system: UVB filter (Octocrylene), UVA filters (Mexoryl SX, Mexoryl XL, Parsol 1789), TiO^2 allows the formula to be photostable.
30 minutes after the application (2 mg/cm^2) of either the broad spectrum sunscreen or its vehicle control, the volar side of the forearms of 4 human volunteers were irradiated with UV-light (2 MED) using a solar simulator. 24 hours after irradiation, sunction blister cups were placed on the test areas test areas and by appling a negative pressure, the formation of suction blisters was induced. Using a specific ELISAs blister fluids were analysed for IL-10 and αMSH. Total mRNA was isolated from the blister roofs, reversed transcripted and the resulting cDNA was used for RT-PCR using primers specific for IL-10, αMSH and β -actin.
Whereas, in the vehicle controlled area, the formation of erythema clearly was visible, it was suppressed on the broad spectrum treated area. Moreover, in comparison to untreated skin, IL-10 and αMSH expression were significantly upregulated in UV-irradiated skin both at the protein and mRNA level. Upon treatment with the broadspectrum sunscreen, the αMSH and IL-10 levels in the suction blister fluids were decreased in comparison to the untreated control area. Similarly, mRNA expression of IL-10 and αMSH was downregulated when compared to untreated irradiated skin.
These data provide first evidence for induction of immunosuppressive mediators in vivo in the skin upon irradiation with UV-light. In addition, there is evidence that the use of a highly effective sunscreen covering the entire UV spectrum (UVB + UVA) can inhibits the UV-mediated induction of these suppressor factors and thereby may prevent local UV-induced immunosuppression.

UV-INDUCED PRODUCTION OF IMMUNOSUPPRESSIVE MEDIATORS IN HUMAN SKIN: PREVENTION BY A BROADSPECTRUM SUNSCREEN

DH. KALDEN [1], T. BRZOSKA [1], T. SCHWARZ [1], A. RICHARD [2], A. ROUGIER [2], TA. LUGER [1]

[1] Ludwig Boltzmann Institute of Cell- and Immunobiology of the Skin, University of Münster, Münster, GERMANY - [2] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE

INTRODUCTION

Irradiation of the skin with UV-light is well known to cause local as well as systemic immunosuppression. Therefore, the effect of UV-irradiation on the production of immunosuppressive mediators Interleukin-10 (IL-10) and α-melanocyte stimulating hormone (α-MSH) was investigated. In addition, the protective effect of a broadspectrum sunscreen on the synthesis and release of these mediators was tested.

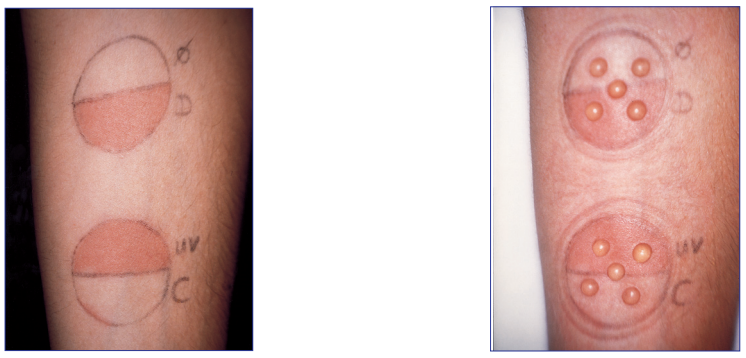
MATERIAL AND METHOD

UV-irradiation: healthy volunteers (n=8) were irradiated on the volar side of the forearms with UV-light (2 MED) using a solar simulator. The irradiated area was either left untreated or treated with a broadspectrum sunscreen (C) or its vehicle control (D). In addition samples were taken from non-irradiated sites.
Composition of product C: Octocrylene, titanium oxide, butyl methoxydibenzoylmethane, drometrizole trisiloxane, terephthalylidene dicamphor sulfonic acid.

Generation of suction blisters: 24h after irradiation suction blister cups were placed on the test areas and formation of suction blisters was induced by using a vacuum pump. Thereafter interstitial fluid was isolated from suction blisters and RNA was harvested from the suction blister roofs.

Semiquantitative RT-PCR: total RNA was isolated from the suction blister roofs using the TRIZOL-method. The RNA was reverse transcribed and subjected to PCR using specific primers for β-Actin, IL-10 and the α-MSH precursor Proopiomelanocortin (POMC).
α-MSH and IL-10 levels in the blister fluid were determined by ELISA.

RESULTS



Figures 1 and 2:

Formation of erythema after UV-irradiation only (UV) and after pretreatment with pretreatment with C (broadspectrum sunscreen) and D (vehicle). Generation of stable suction blisters in the tested areas.

The volar sides of the forearms were irradiated with UV-light (2 MED) resulting in the induction of erythema. However, the tested sunscreen was able to prevent erythema formation, whereas the vehicle did not protect (Figure 1). Suction blisters were generated 24h after treatment. The resulting blisters were stable for an extended period of time and contained a clear liquid (Figure 2).

Expression of α-MSH and IL-10 in human skin

Basal levels of the immunosuppressor IL-10 and the neuropeptide α-MSH were detected in similar amounts in untreated skin samples. Irradiation with UVB-light led to a significant increase of both mediators (Table 1).

	IL-10 (pg/ml)	α-MSH (pg/ml)
Control	16.13 ± 6.84	16.57 ± 9.22
UV	49.63 ± 21.94	45.00 ±18.37

Table 1:

Amounts of a-MSH and IL-10 in suction blister fluids 24h after UV-irradiation in comparison to not irradiated control.

Effect of a broadspectrum sunscreen on the UVB-induced expression of α-MSH and IL-10

POMC, the precursor molecule for α-MSH, is expressed in the blister fluid of untreated human skin. In UV-irradiated skin a significant upregulation of POMC mRNA expression was detected. UV-induced POMC expression was downregulated by the sunscreen C. As expected, treatment with the vehicle only was not able to inhibit the UV-induced POMC expression (Figure 3).

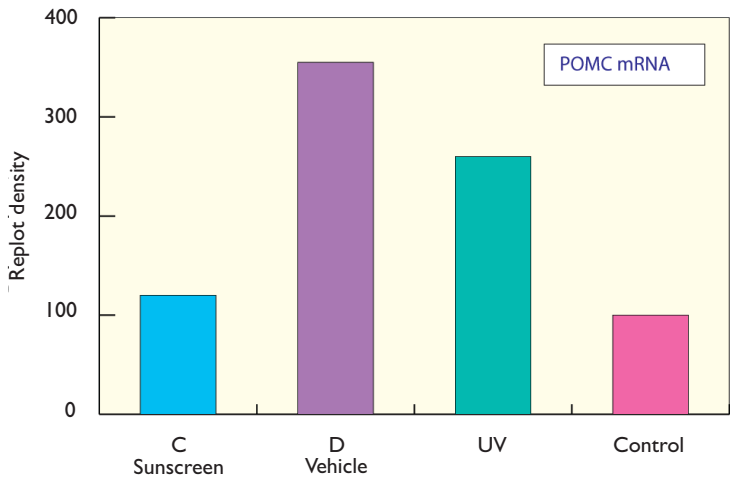


Figure 3:

POMC mRNA expression in human skin 24h after treatment with sunscreen C followed by UV-irradiation in comparison to untreated control. Results are expressed as the mean of 3 different experiments.

The amount of α-MSH in the suction blister fluid is significantly increased 24h after UV-irradiation. Pretreatment with sunscreen C led to a reduction of the α-MSH level in comparison to UV-light only (Figure 4).
The IL-10 concentration in the blister fluids was strongly elevated upon UV-irradiation. Pretreatment with vehicle only did not influence the UV-induced IL-10 expression, whereas treatment with sunscreen C was able to prevent UV-mediated IL-10 production (Figure 5).

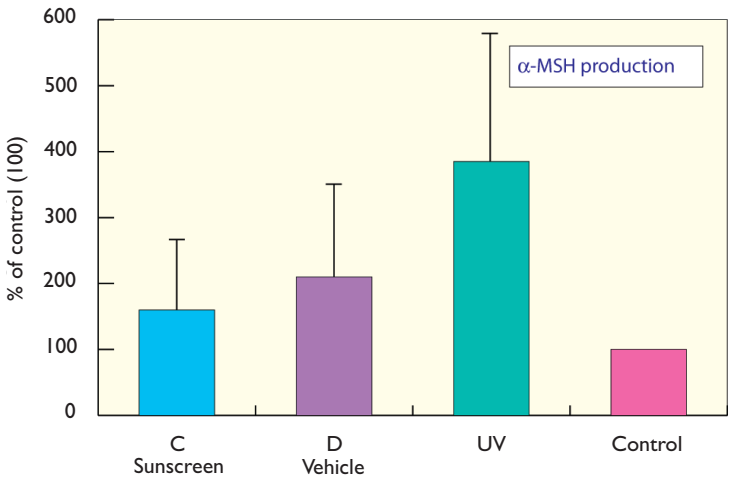


Figure 4:

Production of α-MSH 24h after treatment in human skin blisters detected by ELISA. Results are indicated in percent of the control α-MSH production (100%).

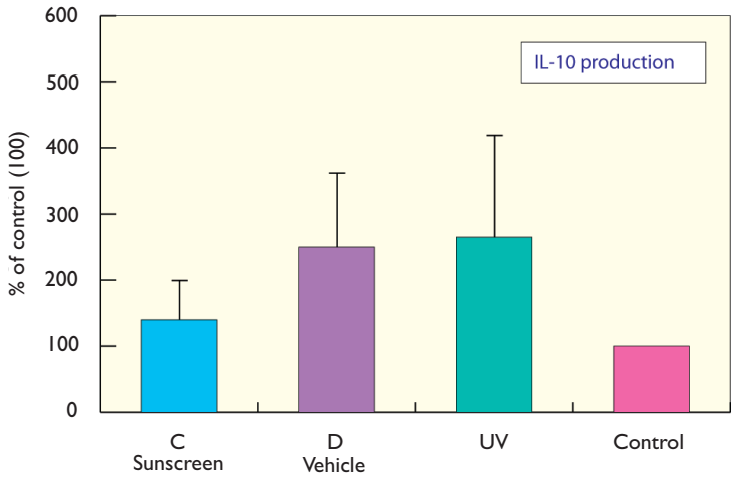


Figure 5:

Detection of IL-10 in human skin blisters 24h after treatment by ELISA. Total amounts of IL-10 were normalized to the control IL-10 production (100%).

CONCLUSION

Our data indicate:
(1) The immunomodulators α-MSH and IL-10 were detected in human skin suction blisters, at the mRNA as well as at the protein level. (2) UV-irradiation significantly upregulated α-MSH and IL-10 synthesis and release. (3) The tested sunscreen C was able to protect erythema formation. (4) Sunscreen C significantly prevented UV-induced POMC expression and IL-10 production.
These data provide first evidence for induction of immunosuppressive mediators *in vivo* in the skin upon irradiation with UV-light. In addition, there is evidence that the use of a highly effective sunscreen covering the entire UV spectrum (UVB + UVA) inhibits the UV-mediated induction of these suppressor factors and thereby may prevent local UV-induced immunosuppression.

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

EVALUATION OF THE CAPACITY OF SUNSCREENS TO PHOTOPROTECT LUPUS ERYTHEMATOSUS PATIENTS BY EMPLOYING THE PHOTOPROVOCATION TEST

H. STEGE [1], M.A. BUDDE [1], S. GRETHER-BECK [1], A. RICHARD [2], A. ROUGIER [2] and J. KRUTMANN [1]

[1] *Clinical and Experimental Photodermatology, Department of Dermatology, Heinrich-Heine-University Düsseldorf, GERMANY* - [2] *La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE*

INTRODUCTION

Lupus erythematosus (LE) is an autoimmune disease that is triggered and exacerbated by ultraviolet (UV) radiation (1). As a consequence, photoprotection is one of the fundamental measures in the management of LE patients (2). In principal two possibilities exist, topical protection using sunscreens or systemic protection employing drugs such as chloroquine, hydroxychloroquine or retinoids. Topical protection with sunscreens has the advantage that unwanted side effects are exclusively local and limited to the rare occurence of photoxic or photoallergic reactions. Although the regular use of sunscreens is routinely recommended to each LE patient, a systematic examination of the efficacy of sunscreens to photoprotect LE patients has not yet been performed.

It has previously been demonstrated that it is possible to induce LE-specific skin lesions in previously non-lesional skin by exposing them to UV radiation under standardized conditions (3, 4). These photoprovocation studies have revealed that (i) the UV radiation spectrum LE patients are sensitive to, includes the UVB (290-320 nm) and/or UVA (320-400 nm) range, and (ii) that the development of irradiation-induced skin lesions does not occur immediately, but requires up to three weeks post irradiation. The availability of standardized photoprovocation protocols provides the unique possibility to test the efficacy of a given sunscreen to photoprotect LE patients under highly standardized and reproducible conditions. In the present study, three commercially available sunscreens were evaluated for their capacity to prevent the development of photoprovocation-induced skin lesions in LE patients.

MATERIAL AND METHOD

Patients

Eleven patients (9 males, 2 females) with photosensitive LE were enrolled after written informed consent was obtained. The average age was 51 years (range 31 to 66 years). Based on clinical, histological and immunofluorescence features they were diagnosed as having subacute cutaneous LE (SCLE; n = 8) or chronic discoid LE (DLE; N = 3). According to previous testing, skin lesions could be induced in all patients upon photoprovocation with a combination of UVA plus UVB, as described below. Criteria for a positive provocative phototest result required that induced lesions clinically resembled LE, histopathologic findings were compatible with LE, and skin lesions developed slowly and persisted for several days.

Sunscreens

Three different sunscreens were used. Sunscreen A (UVB: Octocrylene; UVA: Mexoryl SX, Mexoryl XL, Parsol 1789; TiO_2) had a SPF > 60, sunscreen B (UVB: Eusolex 6300, Parsol MCX, Uvinul T150, Neohelipan; UVA: Parsol 1789; TiO_2) had a SPF > 75, and sunscreen C (Eusolex 6300, Parsol MCX, Uvinul T150; UVA: Parsol 1789; TiO_2) had a SPF = 35. Each sunscreen was applied 20 minutes prior to irradiation on a given test area of 5 x 5 cm according to the COLIPA norm.

Photoprovocation testing

The light sources used were a Sellamed Dr. Sellmeier partial body irradiation device (340-400 nm; Sellas GmbH) for UVA testing, and a UV-800 unit lamp with fluorescent bulbs (285-350 nm) Philips TL 20 W/12 (Waldmann) for UVB testing. Irradiation output was monitored by means a UV radiometer (Mutzhas UVA METER) and Waldmann UV-spectrometer. For the provocative phototest, four areas of 5 x 5 cm of uninvolved skin on the back were irradiated depending on the patient's history and previous phototesting results with single doses of 100 J UVA per cm^2 with or without an additional exposure to 1.5 MED of UVB radiation daily for three consecutive days. Test areas were evaluated until specific lesions appeared for up to 4 weeks after the last session of irradiation. None of the patients received any medication during the testing period.

RNA extraction and RT-PCR analysis

In 3 of 11 patients, 4 mm punch biopsy specimens were obtained from unirradiated control skin, from an unprotected and from a sunscreen pretreated photoprovocation test site 1 week after the last irradiation. Intercellular adhesion molecule-1 (ICAM-1) mRNA expression was assessed in a semiquantitative manner by differential RT-PCR. This method has previously been used for analysis of in-situ expression of specific mRNAs and found to be highly sensitive and reliable (5). Each PCR of each sample was carried out at least two times. Products were visualized by gel analysis using ethidium bromide staining.

CONCLUSION

In the present study, we have assessed the efficacy of three different, commercially available sunscreens to prevent the UV radiation-induced generation of skin lesions in photosensitive LE patients by employing a standard provocative phototest. All patients tested had a history of positive photoprovocation testing using this standard protocol. In the present study, positive photoprovocation results were obtained in 100% of tested patients, indicating that the photoprovocation protocol used is characterized by a high intraindividual reproducibility. This indicates that the provocative phototest is a reliable *in vivo* assay which can be used to assess the efficacy of photoprotective measures in LE patients under standardized, controlled and reproducible conditions. When tested in this assay, three different, commercially available sunscreens were found to prevent the development of photoprovocation-induced skin lesions in these patients. These observations demonstrate that the use of sunscreens is beneficial for LE patients. But the capacity of the three sunscreens to provide protection varied markedly. In all patients tested, skin lesions had been provoked by employing a combination of UVB and UVA radiation devices. The sunscreens tested had a similar capacity to protect against UVB radiation, as indicated by their SPF. Variations in the efficacy to photoprotect LE patients, as observed in this study, might thus reflect differences in the capacity of the three tested sunscreens to protect against UVA radiation. Accordingly, all three sunscreens employed Parsol 1789 as a UVA-filter, but sunscreen A additionally contained a combination of Mexoryl SX plus Mexoryl XL. It was the latter sunscreen that provided complete protection in 100% of tested patients and thus was clearly superior to the other sunscreens, which protected in 45 and 27%, respectively. Taken together these observations indicate that (i) sunscreens are capable of preventing the development of skin lesions in photosensitive LE patients and that (ii) the efficacy of a given sunscreen to provide photoprotection to LE patients depends on the type of UV filter employed.

Epidermal keratinocytes in LE-specific skin lesions have been shown to express the adhesion molecule ICAM-1, which is known to be functionally involved in the interaction of keratinocytes with skin-infiltrating T-cells (6). In the present study, we have observed that in photoprovocation-induced LE skin lesions, similar to genuine skin lesions from LE patients, increased ICAM-1 expression can be observed. Increased ICAM-1 expression was found to precede the development of clinically apparent skin lesions by at least 1 to 2 weeks. In addition, upregulation of ICAM-1 expression could be completely prevented through the application of sunscreen A, thus corroborating and extending the clinical observation that this sunscreen was highly effective in providing photoprotection in LE patients. These observations also indicate that UV radiation-induced upregulation of keratinocyte ICAM-1 expression might be related to the pathogenesis of skin lesions in LE patients (7 ,8).

RESULTS

Photoprovocation testing was positive in all patients assessed. All sunscreens tested provided protection against the development of UV radiation-induced skin lesions in these patients. This protective capacity, however, markedly varied between the three sunscreens tested (Figures 1a, 1b). Sunscreen A provided complete protection in 11 out of 11 patients, sunscreen B in 5 out of 11 patients, and sunscreen C in 3 out of 11 patients.

Figure 1a:

Photoprovocation test reactions 2 weeks after the 3rd irradiation with a combination of UVB plus UVA in a patient with lupus erythematosus (overview and details). Prior to irradiation, test sites were either pretreated with sunscreens A, B or C or left unprotected (=unbehandelt; D).

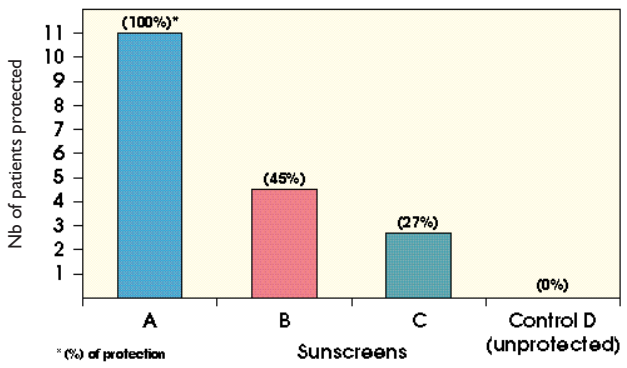


Figure 1b:

Photoprovocation test reactions 2 weeks after the 3rd irradiation with a combination of UVB plus UVA in a patient with lupus erythematosus. Prior to irradiation, test sites were either sunscreens A, B or C or left unprotected (D).

Development of skin lesions in irradiated skin areas was associated with an increased expression of keratinocyte ICAM-1 mRNA expression (Figure 2). Application of sunscreen A to test areas prior to photoprovocation testing completely prevented not only the development of skin lesions, but also the increase in ICAM-1 mRNA expression (Figure 3).

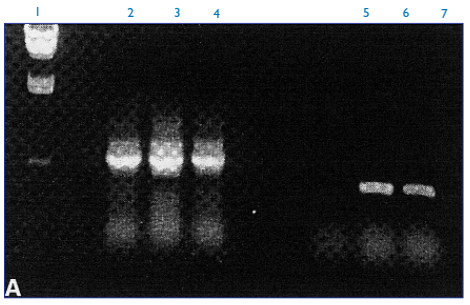


Figure 2:

Semiquantitative RT-PCR for ICAM-1 mRNA expression in photoprovocation test areas 1 week after the 3rd irradiation of a patient with lupus erythematosus. Biopsies were obtained from unirradiated control skin (no UVA), unprotected, UV-irradiated skin (UVA) or sunscreen A-pretreated, UV-irradiated skin. ICAM-1 mRNA expression was assessed by semiquantitative RT-PCR as described in Material and Method. A: Ethidium bromide gel analysis. Lane 1: lambda HindIII standard, lane 2: ß-actin expression in unirradiated control skin; lane 3: ß-actin expression in unprotected, UV-irradiated skin; lane 4: ß-actin expression in sunscreen A-pretreated, UV-irradiated skin; lane 5: ICAM-1 mRNA expression in unirradiated control skin; lane 6: ICAM-1 mRNA expression in unprotected, UV-irradiated skin; lane 7: ICAM-1 mRNA expression in sunscreen A-pretreated, UV-irradiated skin. B: Summary of RT-PCR results from three patients. ICAM-1 mRNA expression in UV-irradiated (UV) or sunscreen A-pretreated, UV-irradiated skin (UV, cream A) is given in fold expression as compared with expression in unprotected, unirradiated skin, which was arbitrarily set as 1.

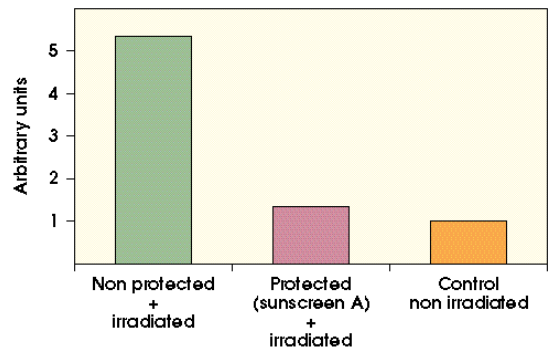


Figure 3:

ICAM-1 mRNA expression in sunscreen protected and unprotected skin areas.

REFERENCES

1. Sontheimer RD, Provost TT. Lupus erythematosus. In: *Sontheimer RD, Provost TT, eds*. Cutaneous manifestations of rheumatic diseases. Baltimore, *Wilkins & Wilkins*, 1-71 (1996)
2. Sontheimer RD. Lupus erythematosus. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB, *eds*. *Fitzpatrick's dermatology in general medicine*, New York: McGraw-Hill 1993-2022 (1999)
3. Lehmann P, Hölzle E, Kind P, Goerz G, Plewig G. Experimental reproduction of skin lesions in lupus erythematosus. *J Am Acad Dermatol*, **22**:181-187 (1990)
4. Walchner M, Messer G, Kind P. Phototesting and photoprotection in LE. *Lupus*, **6**:167-174(1997) **63**:583-594 (1996)
5. Grewe M, Gyufko K, Schöpf E, Krutmann J. Lesional expression of interferon-γ in atopic eczema. *Lancet* **343**:25-26 (1994)
6. Tebbe B, Mazur L, Staddler R, Orfanos CE. Immunohistochemical analysis of chronic discoid and subacute cutaneous lupus erythematosus - relation to immunopathologic mechanisms. *Br J Dermatol*, **132**:25-31 (1995)
7. Norris DA. Photoimmunology of lupus erythematosus. In: Krutmann J, Elmets CA, *eds*. *Photoimmunology*, Oxford:Blackwell, 209-227 (1995)
8. Sontheimer RD. Photoimmunology of lupus erythematosus and dermatomyositis: a speculative review. *Photochem Photobiol*, **63**:583-594 (1996)

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

COMPARISON OF UVA PROTECTION AFFORDED BY SUNSCREENS WITH HIGH SUN-PROTECTION FACTOR

D. MOYAL [1], L. DUTEIL [2], C. QUEILLE-ROUSSEL [2], J.P. ORTONNE [2], A. RICHARD [3], A. ROUGIER [3]

[1] L'ORÉAL Recherche, Clichy, FRANCE - [2] CPCAD, Nice, FRANCE - [3] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE

INTRODUCTION

UVA have been recognized to be involved in the induction of solar elastosis and have been shown to play a determinant role in photo-sensitization, different photo-induced dermatosis, photoimmunosuppression and skin cancer. The evaluation of UVA protection afforded by a sunscreen is therefore of premium importance.
In the present study the persistent pigment darkening reaction induced by UVA was used to compare the protection afforded by 9 commercially available sunscreens with sun protection factors (SPF) ranging from 25 to 100 and containing UVA filters and/or physical agents.

MATERIAL AND METHOD

Tests products
Nine commercially available sunscreens with an SPF between 25 and 100 that claimed to offer UVA and UVB protection were selected. They contain different combinations of UVA filters.

Codage	UVA filtration	Labelled SPF
A	Avobenzone, TiO_2	SPF 25
B	TiO_2, ZnO	SPF 50
C	TiO_2	SPF 25
D	TiO_2, ZnO	
E	Tinosorb M, TiO_2	SPF 60
F	Mexoryl® SX and XL, Avobenzone, TiO_2	SPF 60+
G	Avobenzone, Tinosorb M	SPF 100
H	Tinosorb M, TiO_2, ZnO	SPF 60
I	Avobenzone, TiO_2, ZnO	SPF 35

Subjects
2 groups of 10 volunteers with skin phototype III or IV with no history of sun or artificial light exposure on their back for at least 3 months.

Application
Application of each product, 15 min before exposure, at a rate of 2 mg/cm² for the first group and 1 mg/cm² for the second group on a surface of 4.5 cm x 4.5 cm on the back.

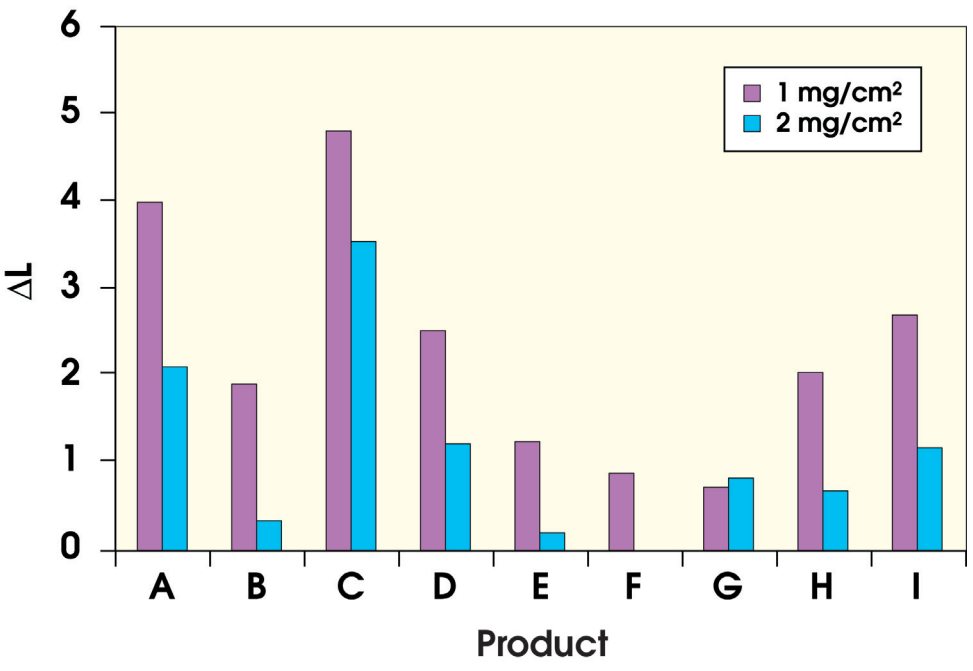
UV source
Xenon Arc lamp 1000 W equipped with a WG335/3 mm and UG11/1 mm thick filters in order to obtain a UVA emission spectrum from 320 nm to 400 nm.
Single exposure at 100 J/cm² on each treated area on a surface of 3 cm diameter.
The photo-unstability of the sunscreen formulations was taken into account by using the UVA dose of 100 J/cm².

Clinical assessment and colorimetric measurements of the pigmentation intensity
Two hours after UVA exposure, the pigmentation intensity was graded visually on a scale of 0 to 10 and measured with a colorimeter (Minolta CR200) in the L a b mode (CIE, 1976). The pigmentation intensity of each area was calculated by subtracting the L (luminance) value of the exposed site from the L value of the adjacent skin.

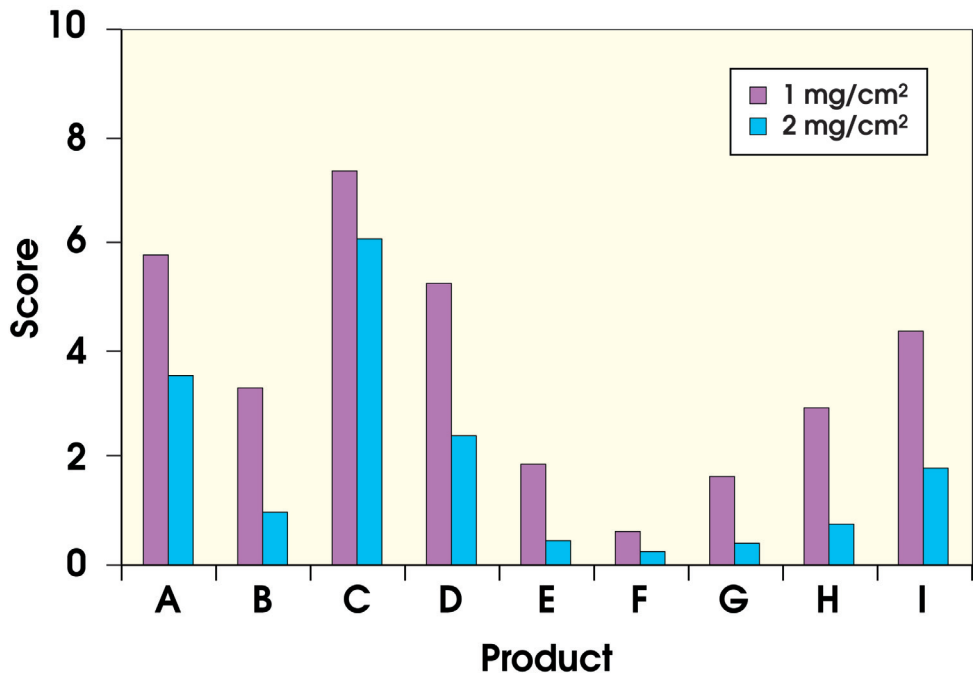
RESULTS

For visual and colorimetric assessment differences were observed between the various sunscreens. These differences were more pronounced at 1 mg/cm².
At this rate, pigmentation intensity was lowest for sunscreen F followed in order of increasing pigmentation by sunscreens G, E, B, H, I, D, A, C.

Pigmentation intensity (DL) with the colorimetric method



Pigmentation intensity with the visual method



The difference in pigmentation intensity at 1 mg/cm² between sunscreen F and all other sunscreens was statistically significant ($p<0.05$) except for sunscreen G ($p=0.09$).

CONCLUSION

These evaluations showed significant differences between the various sunscreens in UVA-induced pigmentation, mainly at the more realistic application rate of 1 mg/cm². Products having the same SPF can show different level of UVA pigmentation prevention thus demonstrating that labeled SPF is not predictive of UVA protection. Products claiming UVA protection are not all equivalent under UVA exposure. A standardized quantitative method could help the consumer in sunscreen selection.

REFERENCES

Moyal D, Chardon A, Kollias N. UVA protection efficacy of sunscreens can be determined by the persistent pigment darkening (PPD) method. *Photodermatol Photoimmunol Photomed*, **16**: 250-255 (2000)

Bissonnette R, Allas S, Moyal D, Provost N. Comparison of UVA protection afforded by high sun protection factor sunscreens. *J Am Acad Dermatol*, **43**:1036-1038 (2000)

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

EVALUATION OF THE CAPACITY OF SUNSCREENS TO PHOTOPROTECT LUPUS ERYTHEMATOSUS PATIENTS BY EMPLOYING THE PHOTOPROVOCATION TEST

H. Stege [1], M.A. Budde [1], S. Grether-Beck [1], A. Richard [2], A. Rougier [2] and J. Krutmann [1].

[1] Clinical and Experimental Photodermatology, Department of Dermatology, Heinrich-Heine-University Düsseldorf, GERMANY.
[2] La Roche-Posay, Pharmaceutical Laboratories, Asnières, FRANCE.

INTRODUCTION

Lupus erythematosus (LE) is an autoimmune disease that is triggered and exacerbated by ultraviolet (UV) radiation (1). As a consequence, photoprotection is one of the fundamental measures in the management of LE patients (2). In principal two possibilities exist, topical protection using sunscreens or systemic protection employing drugs such as chloroquine, hydroxychloroquine or retinoids. Topical protection with sunscreens has the advantage that unwanted side effects are exclusively local and limited to the rare occurence of photoxic or photoallergic reactions. Although the regular use of sunscreens is routinely recommended to each LE patient, a systematic examination of the efficacy of sunscreens to photoprotect LE patients has not yet been performed.
It has previously been demonstrated that it is possible to induce LE-specific skin lesions in previously non-lesional skin by exposing them to UV radiation under standardized conditions (3, 4). These photoprovocation studies have revealed that (i) the UV radiation spectrum LE patients are sensitive to includes the UVB (290-320 nm) and/or UVA (320-400 nm) range, and (ii) that the development of irradiation-induced skin lesions does not occur immediately, but requires up to three weeks post irradiation. The availability of standardized photoprovocation protocols provides the unique possibility to test the efficacy of a given sunscreen to photoprotect LE patients under highly standardized and reproducible conditions. In the present study, three commercially available sunscreens were evaluated for their capacity to prevent the development of photoprovocation-induced skin lesions in LE patients. We have found that all three sunscreens were effective in providing photoprotection, however, to various extents.

MATERIALS AND METHODS

Patients:

Eleven patients (9 male, 2 female) with photosensitive LE were enrolled after written informed consent was obtained. The average age was 51 years (range 31 to 66 years). Based on clinical, histological and immunofluorescence features they were diagnosed as having subacute cutaneous LE (SCLE; n = 8) or chronic discoid LE (DLE; N = 3). According to previous testing, skin lesions could be induced in all patients upon photoprovocation with a combination of UVA plus UVB, as described below. Criteria for a positive provocative phototest result required that induced lesions clinically resembled LE, histopathologic findings were compatible with LE, and skin lesions developed slowly and persisted for several days.

Sunscreens:

Three different sunscreens were used. Sunscreen A (UVB: Octocrylene; UVA: Mexoryl SX, Mexoryl XL, Parsol 1789; TiO_2) had a SPF > 60, sunscreen B (UVB: Eusolex 6300, Parsol MCX, Uvinul T150, Neohelipan; UVA: Parsol 1789; TiO_2) had a SPF > 75, and sunscreen C (Eusolex 6300, Parsol MCX, Uvinul T150; UVA: Parsol 1789; TiO_2) had a SPF = 35. Each sunscreen was applied 20 minutes prior to irradiation in a given test area of 5 x 5 cm according to the COLIPA norm.

Photoprovocation testing:

The light sources used were a Sellamed Dr. Sellmeier partial body irradiation device (340-400 nm; Sellas GmbH, Gevelsberg, Germany) for UVA testing, and a UV-800 unit lamp with fluorescent bulbs (285-350 nm) Philips TL 20 W/12 (Waldmann, Villingen-Schwenningen, Germany) for UVB testing. Irradiation output was monitored by means of an UV radiometer (Mutzhas UVAMETER, Munich, Germany) and Waldmann UV-spectrometer. For the provocative phototest, four areas of 5 x 5 cm of uninvolved skin on the back were irradiated depending on the patient's history and previous phototesting results with single doses of 100 J UVA per cm^2 with or without an additional exposure to 1.5 MED of UVB radiation daily for three consecutive days. Test areas were evaluated until specific lesions appeared for up to 4 weeks after the last session of irradiation. None of the patients received any medication during the testing period.

RNA extraction and RT-PCR analysis:

In 3 of 11 patients, 4 mm punch biopsy specimens were obtained from unirradiated control skin, from an unprotected and from a sunscreen pretreated photoprovocation test site 1 week after the last irradiation. Intercellular adhesion molecule-1 (ICAM-1) mRNA expression was assessed in a semiquantitative manner by differential RT-PCR. This method has previously been used for analysis of in-situ expression of specific mRNAs and found to be highly sensitive and reliable (5). Each PCR of each sample was carried out at least two times. Products were visualized by gel analysis using ethidium bromide staining.

DISCUSSION

In the present study, we have assessed the efficacy of three different, commercially available sunscreens to prevent the UV radiation-induced generation of skin lesions in photosensitive LE patients by employing a standard provocative phototest. All patients tested had a history of positive photoprovocation testing using this standard protocol. In the present study, positive photoprovocation results were obtained in 100% of tested patients, indicating that the photoprovocation protocol used is characterized by a high intraindividual reproducibility. This indicates that the provocative phototest is a reliable *in vivo* assay which can be used to assess the efficacy of photoprotective measures in LE patients under standardized, controlled and reproducible conditions.
When tested in this assay, three different, commercially available sunscreens were found to prevent the development of photoprovocation-induced skin lesions in these patients. These observations demonstrate that the use of sunscreens is beneficial for LE patients. In the present study, the capacity of the three sunscreens to provide protection varied markedly.
In all patients tested, skin lesions had been provoked by employing a combination of UVB and UVA radiation devices. The sunscreens tested had a similar capacity to protect against UVB radiation, as indicated by their SPF. Variations in the efficacy to photoprotect LE patients, as observed in this study, might thus reflect differences in the capacity of the three tested sunscreens to protect against UVA radiation. Accordingly, all three sunscreens employed Parsol 1789 as a UVA-filter, but sunscreen A additionally contained a combination of Mexoryl SX plus Mexoryl XL. It was the latter sunscreen that provided complete protection in 100% of tested patients and thus was clearly superior to the other sunscreens, which protected in 45 and 27%, respectively. Taken together these observations indicate that (i) sunscreens are capable of preventing the development of skin lesions in photosensitive LE patients and that (ii) the efficacy of a given sunscreen to provide photoprotection to LE patients depends on the type of UV filter employed.
Epidermal keratinocytes in LE-specific skin lesions have been shown to express the adhesion molecule ICAM-1, which is know to be functionally involved in the interaction of keratinocytes with skin-infiltrating T-cells (6). In the present study, we have observed that in photoprovocation-induced LE skin lesions, similar to genuine skin lesions from LE patients, increased ICAM-1 expression can be observed. Increased ICAM-1 expression was found to precede the development of clinically apparent skin lesions by at least 1 to 2 weeks. In addition, upregulation of ICAM-1 expression could be completely prevented through the application of sunscreen A, thus corroborating and extending the clinical observation that this sunscreen was highly effective in providing photoprotection in LE patients. These observations also indicate that UV radiation-induced upregulation of keratinocyte ICAM-1 expression might be related to the pathogenesis of skin lesions in LE patients (7 ,8).

REFERENCES

1 - Sontheimer RD, Provost TT. Lupus erythematosus. In: Sontheimer RD, Provost TT, eds. Cutaneous manifestations of rhemumatic diseases. Baltimore, Wilkins & Wilkins, 1996:1-71.
2 - Sontheimer RD. Lupus erythematosus. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB, eds. Fitzpatrick's dermatology in general medicine. New York: McGraw-Hill 1999:1993-2022.
3 - Lehmann P, Hölzle E, Kind P, Goerz G, Plewig G. Experimental reproduction of skin lesions in lupus erythematosus. J Am Acad Dermatol 1990:22:181-187.
4 - Walchner M, Messer G, Kind P. Phototesting and photoprotection in LE. Lupus 1997:6:167-174.
5 - Grewe M, Gyufko K, Schöpf E, Krutmann J. Lesional expression of interferon-γ in atopic eczema. Lancet 1994:343:25-26.
6 - Tebbe B, Mazur L, Staddler R, Orfanos CE. Immunohistochemical analysis of chronic discoid and subacute cutaneous lupus erythematosus - relation to immunoptahologic mechanisms. Br J Dermatol 1995:132:25-31.
7 - Norris DA. Photoimmunology of lupus erythematosus. In: Krutmann J, Elmets CA, eds. Photoimmunology. Oxford: Blackwell, 1995:209-227.
8 - Sontheimer RD. Photoimmunology of lupus erythematosus and dermatomyositis: a speculative review. Photochem Photobiol 1996:63:583-594.

RESULTS

Photoprovocation testing was positive in all patients assessed. All sunscreens tested provided protection against the development of UV radiation-induced skin lesions in these patients. This protective capacity, however, markedly varied between the three sunscreens tested (figures 1a, 1b). Sunscreen A provided complete protection in 11 out of 11 patients, sunscreen B in 5 out of 11 patients, and sunscreen C in 3 out of 11 patients.

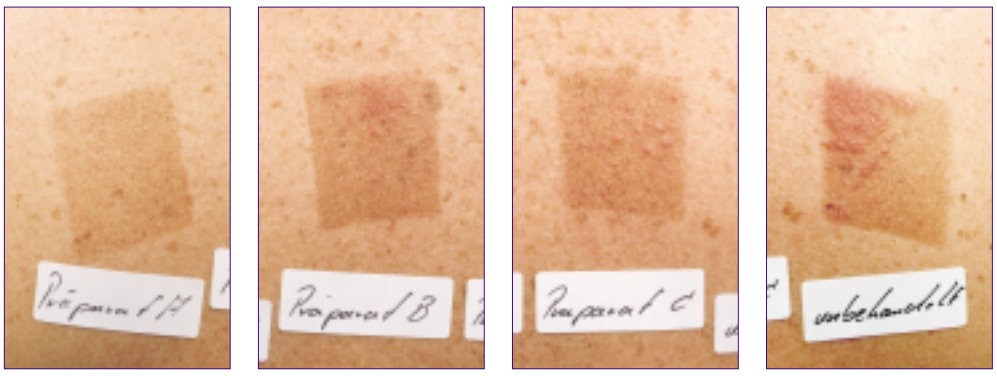


Fig. 1a:
Photoprovocation test reactions 2 weeks after the 3rd irradiation with a combination of UVB plus UVA in a patient with lupus erythematosus (overview and details). Prior to irradiation, test sites were either pretreated with sunscreens A, B or C or left unprotected (=unbehandelt; D).

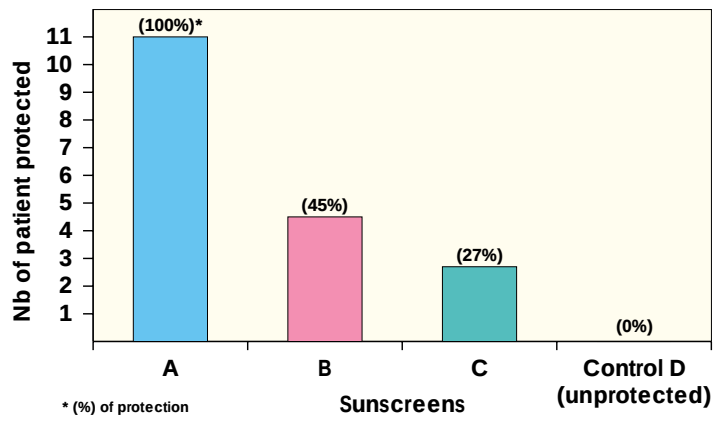


Fig. 1b:
Photoprovocation test reactions 2 weeks after the 3rd irradiation with a combination of UVB plus UVA in a patient with lupus erythematosus. Prior to irradiation, test sites were either sunscreens A, B or C or left unprotected (D).

Development of skin lesions in irradiated skin areas was associated with an increased expression of keratinocyte ICAM-1 mRNA expression (figure 2). Application of sunscreen A to test areas prior to photoprovocation testing completely prevented not only the development of skin lesions, but also the increase in ICAM-1 mRNA expression (figure 3).

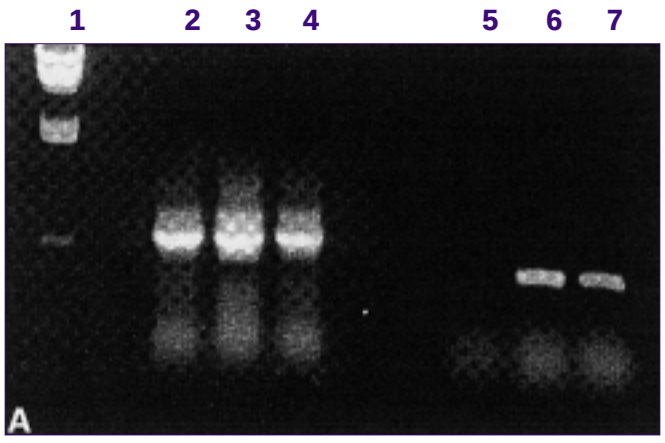


Fig. 2:
Semiquantitative RT-PCR for ICAM-1 mRNA expression in photoprovocation test areas 1 week after the 3rd irradiation of a patient with lupus erythematosus. Biopsies were obtained from unirradiated control skin (no UVA), unprotected, UV-irradiated skin (UVA) or sunscreen A-pretreated, UV-irradiated skin. ICAM-1 mRNA expression was assessed by semiquantitative RT-PCR as described in Materials and Methods. A: Ethidium bromide gel analysis. Lane 1: lambda HindIII standard, lane 2: ß-actin expression in unirradiated control skin; lane 3: ß-actin expression in unprotected, UV-irradiated skin; lane 4: ß-actin expression in sunscreen A-pretreated, UV-irradiated skin; lane 5: ICAM-1 mRNA expression in unirradiated control skin; lane 6: ICAM-1 mRNA expression in unprotected, UV-irradiated skin; lane 7: ICAM-1 mRNA expression in sunscreen A-pretreated, UV-irradiated skin. B: Summary of RT-PCR results from three patients. ICAM-1 mRNA expression in UV-irradiated (UV) or sunscreen A-pretreated, UV-irradiated skin (UV, cream A) is given in fold expression as compared with expression in unprotected, unirradiated skin, which was arbitrarily set as 1.

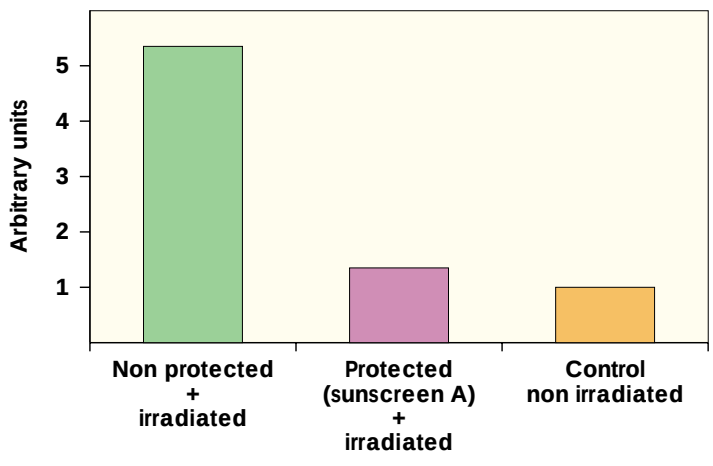


Fig. 3:
ICAM-1 mRNA expression in sunscreen protected and unprotected skin areas.

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

PROTECTIVE EFFECT OF A BROADSPECTRUM UVA-UVB SUNSCREEN IN THE RETINOID THERAPY DURING SUMMER SEASON

H. ZELENKOVA [1], J. STRACENSKA [2], A. RICHARD [3] and A. ROUGIER [3]

[1] Department of Dermatology, Svidnik, SLOVAK REPUBLIC - [2] Department of Dermatology, University of T.G. Masaryk, Brno, CZECH REPUBLIC - [3] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE

OBJECTIVE OF THE STUDY

To verify usage a broadspectrum UVA-UVB sunscreen for targeted skin protection at patients with acne nodulocystica gravis or acne conglobata treated during summer months by Roaccutane® Roche caps.

DEFINITION AND TYPE OF THE PERFORMED STUDY

Type of the study - IV - post-registration observance.
Blind; patients randomised into two groups with and without application of the Anthélios XL60+ cream.

MATERIAL AND METHOD

Division of the patients into two groups:

Group I : application of Anthélios XL60+ cream during the whole term of Roaccutane® caps therapy.
Group II : restricted application of Anthélios XL60+ cream to a specified place at the forearm during one specified week, when observation took place.

Locality of observing the effect of protection against the UV radiation:

Volar area of the left forearm, demarcated area of 5 x 5 cm to observe the effect of applying Anthélios XL60+ cream or a blind excipient.

Prerequisite:

Before assigning any patient into the study, skin photo-type was determined and a photo-test was carried out (to assess degree of the erythema occurring by exposure to the source of UV radiation gluteal area). As a source of radiation in the photo-test carried out, a mercury discharge lamp 125 W (mountain sun) was chosen.

Workplace : Department of Dermatovenerology, Svidnik, Slovak Republic.
Number of patients : 26 (14 males, 12 females)
Average age : men 19,64 year, women 20,5 year
Diagnosis : acne nodulocystica gravis, acne conglobata
Duration of the disease : in average: men 3.57 years, women 3.33 years
Duration of the treatment : in average: men 151 days, women 113 days
Photo-type : I - 4 patients (2 males, 2 females)
II - 20 patients (10 males, 10 females)
III - 2 patients (male)

Inclusion criteria:

- activation of the basic disease, apparent symptoms of bacterial infection (pustules, nodules)
- apparent and severe symptoms corresponding with the assessment according to Cook scale 6-10
- lack of oral treatment by antibiotics, chemotherapeutics or retinoids one month before assignment into the study
- resistance to the treatment applied so far

Exclusion criteria:

- hypersensitivity to components of the products (Roaccutane® Roche, Anthélios XL60+ (La Roche-Posay Pharmaceutical Laboratories), Ceralip (La Roche-Posay Pharmaceutical Laboratories)
- hypersensitivity to parabens
- patients with extremely sensitive skin
- patients under 15 years of age
- pregnancy, lactation

Duration of the study: 4 months
Randomisation : at assignment into the study
Oral drugs used : Roaccutane® Roche caps. 10 and 20 mg
Term of application : 1-3 months, according to individual response
Local agents used : options: Acnefug® EL, Acnefug® liquid N, magistraliter ointments with ichthammol Acidi salicylici, resorcini, or Skinoren® (azelain acid), in justified cases
ointments with antibiotics; Effaclar, Effidrate; soap with Ichthammol SANO 5%, 8% Thermal water La Roche-Posay
Other : mechanical cleaning
Targeted protection against solar radiation : Anthélios XL60+ cream
Special protection lips : Ceralip
Term of application : during usage of Roaccutane® Roche caps.
Recommended personal hygiene agents : non-irritating local agents, not to combine the recommended treatment with other products
Checks : at two-week intervals

RESULTS

Tolerance and adverse effects:

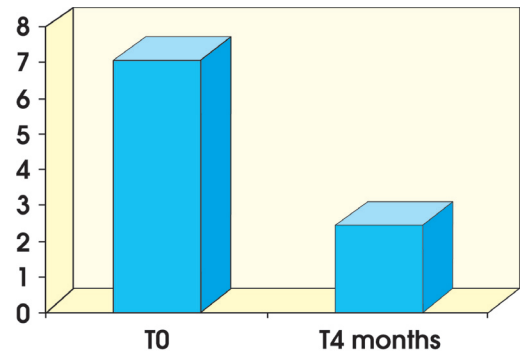
A broadspectrum UVA-UVB sunscreen induces no adverse effects (very good tolerance in all patients).
Patient satisfaction: excellent 96%, very good 4%
Roaccutane® Roche, 4 patients with headache and nausea

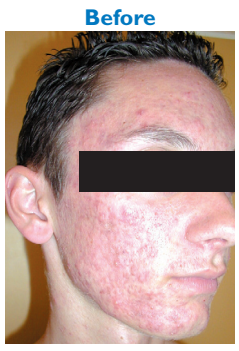
Efficacy:

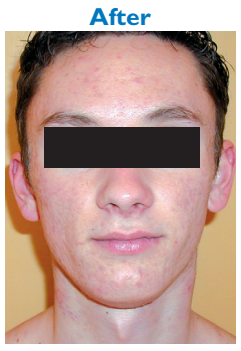
Roaccutane® local finding evaluation (Cook index scale):

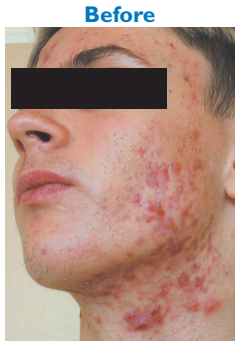
Before treatment:	degree 9 -	3 patients	(1 male, 2 females)
	degree 8 -	8 patients	(4 males, 4 females)
	degree 7 -	4 patients	(3 males, 1 female)
	degree 6 -	11 patients	(6 males, 5 females)
After treatment:	degree 6 -	1 patient	(1 female)
	degree 3 -	11 patients	(6 males, 5 females)
	degree 2 -	14 patients	(8 males, 6 females)

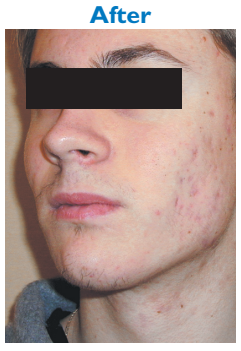
Cook index (mean values)

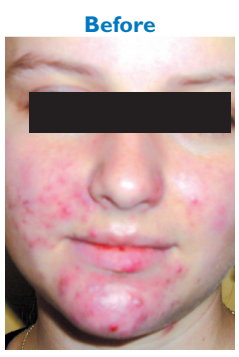


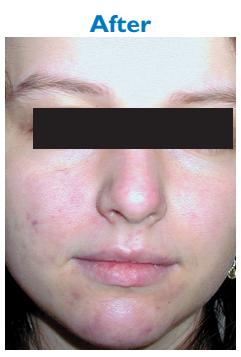












DISCUSSION

The effect of the broadspectrum UVA-UVB sunscreen in the course of the whole treatment by Roaccutane®, has been consonantly assessed both by the dermatologist and the patients as perfect protection (96%). One female patient (who interrupted the treatment) considered its effect as indistinct (4%). Adverse effects such as irritation or agent intolerance were not reported at any patient. Evaluation of the response with a weekly application of a blind excipient at 12 patients (6 females, 6 males) has confirmed in full excellent effects of the broadspectrum UVA-UVB sunscreen. In the group of men, two patients with photo-type I showed an apparent erythema on the third day of placebo application and a burning erythema on the seventh day, in general the patients with photo-type II reported the same result, but the burning was not so distinct. In the group of women, one female patient, who interrupted the treatment, has been excluded from the study. In total, 5 women with placebo application were evaluated (showed the most distinct response there was burning of the treated place on the 7th day), and four with photo-type II. The changes at other women were in general identical with the changes at men. One woman showed basically the same response for the whole week.

CONCLUSION

The need for targeted use of photo protective agents enabling stay at the sun without risk of origination of adverse effects, can be traced back to 1920s. The efforts of research and producers concentrate on preparing such photo protective agents for external use, which would potentiate the influence of natural pigmentation and restrict harmful impact of solar radiation. The majority of the photo protective agents used nowadays is a combination of UVB and UVA filters, often containing also a physical UV-blocker. So these agents are convenient mostly for people with photo-type I and II.
The performed study has in full scope justified the idea, that in case of targeted use of sunscreens with both high SPF and UVA-PF and good cooperation with patients, the trouble-free treatment by systemic retinoids is possible even in summer months.

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

HIGH PROTECTIVE EFFECT OF A BROAD-SPECTRUM SUNSCREEN AGAINST TETRACYCLINES PHOTOTOXICITY

L. Duteil [1], C. Queille-Roussel [1], A. Rougier [2], A. Richard [2], J.P. Ortonne [1].

[1] CPCAD, Hôpital L'Archet 2, Nice, FRANCE.

[2] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE.

INTRODUCTION

The tetracyclines group provides an effective and widely used treatment for acne. The major disadvantage of this treatment is that it express a phototoxicity potential (1,2). Many phototoxic compounds are activated by exposure in the UVA (320-400 nm) domain of the ultraviolet light. However, UVB (280-320 nm) are known to enhance the phototoxic reaction induced by UVA. Therefore, only a highly protective UVA+UVB broad spectrum sunscreen may allow the continuation of tetracycline acne or rosacea treatment during sunny days in summer time.

The aim of the present study was to assess the protective effect of a UVB+UVA high protection sunscreen reinforced in short UVA domain (Anthelios XL, La Roche-Posay) against the phototoxicity of two tetracyclines used to treat acne and rosacea.

MATERIALS AND METHODS

After providing written informed consent, seventeen patients (12 female, 5 male), with skin type II (n=1) and III (n=16), aged from 19 to 60 years (mean age: 31±13 years), treated at least since one week with either doxycycline (N=9) or limecycline (N=8), were included in this study. At Day 1, six zones (1.2 cm^2 in diameter) of the back were irradiated with increasing doses of UVA (from 22 to 70 J/cm^2) in order to determine the Minimal Phototoxic Dose (MPD) in unprotected skin. The Light source was a solar simulator (Idem 3000, Arquanciel, France) equipped with a high pressure vapour xenon lamp filtered with a UG5+WG335 filters combination in order to select only the UVA part of the solar spectrum since the action spectrum of tetracyclines is located within this spectral domain. UVA light intensity was checked prior to each irradiation using a UV dosimeter (3D-600, Solar Light, USA). At Day 2, the MPD was determined using a 0 to 4 clinical score scale (see Table 1). For the positive patients (clinical score 1), a high protection sunscreen (Anthelios XL) and its vehicle were applied (2 mg/cm^2) on either side of the back. Fifteen minutes after application, the treated zones were exposed on three sub-sites using three UVA doses equivalent to: 0.75MPD, 1MPD and 1.25MPD. At Day 3, the exposed zones were assessed by colorimetry (CR300, Minolta) and using the visual scoring system.

0	No erythema
0.5	Doubtful erythema
1	Mild erythema (MPD)
2	Moderate erythema
3	Marked erythema
4	Severe erythema

Table 1: Clinical scoring system

RESULTS

The results indicated that at Day 2, the MPD could be determined on 12 patients out of 17 (MPD mean value = 49±5 J/cm^2). Positive patients were 7 treated with doxycycline (0.1 or 0.2 g/day) and 5 treated with limecycline (0.3 to 0.6 g/day). Clinical evaluations of erythema (Figure 1) revealed that for the 0.75MPD UVA dose, no reaction at all was observed on the protected zone whereas some weak to severe reactions were observed on the vehicle zone. For the 1.25 MPD only four weak erythema (out of 12) were seen on the protected zone whereas 3 weak, 1 moderate and 8 marked reactions were observed on the vehicle zone. Figure 2 illustrates the type of phototoxic reactions observed at Day 3 on the vehicle treated zone and, in comparison, the level of protection afforded by Anthelios XL. Colorimetry coordinates a* (expressing redness of the skin in the L* a* b* system) were measured on the treated and irradiated test zones and on adjacent treated but non irradiated control test zones. Figure 3 illustrates the a* (test zone -control zone) obtained for Anthelios XL and the vehicle for the different UV doses. This figure indicates that 0colorimetry results corroborated the clinical evaluations.

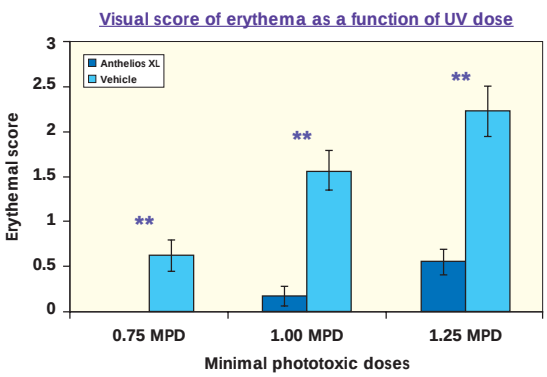


Figure 1:
Visual score of erythema (mean ± sem) assessed 24 hours (Day 3) after UVA irradiation on Anthelios XL and vehicle treated zones.
**** indicates the significance level of product 's comparison (Wilcoxon test, p<0.01).**

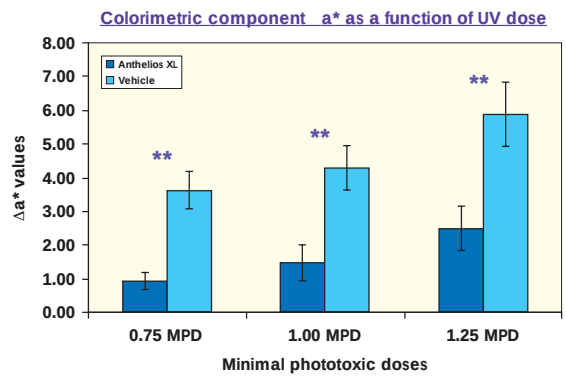


Figure 3:
Colorimetric measurement of erythema (a*, mean ± sem) measured 24 hours (Day 3) after UVA irradiation on Anthelios XL and vehicle treated zones.
**** indicates the significance level of product 's comparison (Student t test, p<0.01).**

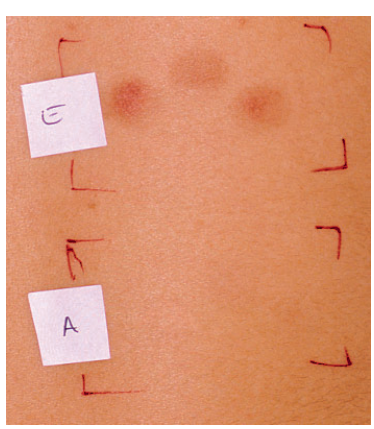


Figure 2:
Photograph of the protected and unprotected irradiated zones at Day 3. Zone A was pretreated with Anthelios XL and zone E with the vehicle)

CONCLUSION

These results indicated that a broad spectrum filtration reinforced in the short UVA domain offers an efficient protection against phototoxicity of tetracyclines treatment, even in conditions of high UVA exposure.

REFERENCES

1. Bjellerup M, Ljunggren. Differences in phototoxic potency should be considered when tetracyclines are prescribed during summer-time. A study on doxycycline and lymecycline in human volunteers, using an objective method for recording erythema. *Br J Dermatol* 1994 Mar;130(3):356-60

2. Hasan T, Kochevar IE, McAuliffe DJ, Cooperman BS, Abdulah D. Mechanism of tetracycline phototoxicity. *Arch Dermatol* 1980 Nov;116(11):1269-71

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE

EVALUATION OF A HIGH SPF, HIGH UVA PF SUNSCREEN IN VITILIGO PATIENTS

G. Leone [1], A. Rougier [2], P. Iacovelli [1], A. Paro Vidolin [1], M. Picardo [1].

[1] Istituto Dermatologico San Gallicano, IRCCS, Roma, ITALY. [2] La Roche-Posay Pharmaceutical Laboratories, Asnières, FRANCE.

INTRODUCTION

The possibility to attenuate the contrast between vitiligo areas and surrounding normal skin is of utmost importance for vitiligo patients especially during summer in the Mediterranean area. This may improve their quality of life considerably. The objective of our study was to evaluate the effectiveness of a high SPF, high UVA PF, broad band, sunscreen, Anthelios XL (SPF 60+ UVA PF 28 PPD), in vitiligo patients during spring and summer.
The effectiveness of the studied sunscreen has been assessed on the basis of its ability to reduce the skin color difference between surrounding normal skin and vitiliginous skin.

Duration of the study: the study began in April 2002 and was concluded in October 2002.

PATIENTS AND METHODS

Patients

Twenty patients with vitiligo have been enrolled: nineteen females and one male, (mean age 42 ± 8 years, range 29-55 years). Seventeen patients (85%) had skin type III (Fitzpatrick) and 3 patients had skin type IV (15%). Fourteen patients presented with stable disease and 6 with active disease. Patients were also screened by means of a questionnaire to assess their sun exposure habits during the summer period.

Inclusion criteria:
- symmetrical vitiligo affecting the face and the dorsa of both hands (constantly photoexposed zones);
- skin type III or IV;
- extensive vitiligo was included as long as the face and the hands were symmetrically affected.

Exclusion criteria:
- treatment with phototherapy or PUVAtherapy during the previous six months;
- face and/or dorsa of the hands totally depigmented
- presence of spontaneous repigmentation;
- age < 18 or > 60 years;
- skin type I, II, V and VI.

Product application

Each patient applied the sunscreen on the face and the back of the hands from April to October. The patients were instructed to apply the sunscreens whenever they stayed outdoors for more than 15 minutes, from 9 a.m. to 7 p.m. When staying outdoors for more than two hours they were instructed to reapply the product every two hours. Reapplication was also recommended after bathing or practicing sports.

Follow up

The patients were visited every two months after the first visit at inclusion (T0). The visits were respectively: T2, T4 and T6.
At each visit the patients have been evaluated clinically and photographs of the treated areas have been taken. Vitiliginous areas have been examined both under natural light conditions and under Wood's light. The presence of erythema or repigmentation on the vitiliginous areas was evaluated. The difference in skin color (pigmentation) between vitiliginous skin and surrounding skin has also been evaluated by means of cutaneous reflectance measurements with a Mexameter MX16 (Courage & Khazaka, Germany) dermaspectrometer. Erythema and pigmentation intensity are expressed respectively as Erythema Index (**EI**) and Melanin Index (**MI**) in arbitrary units.

The photographs have been revised by an independent observer that attributed to each body site, and for each visit, a clinical score taking into account the difference in pigmentation between vitiligo areas and normal skin, as follows: marked difference in pigmentation = 3; moderate difference in pigmentation = 2; slight difference in pigmentation = 1; 0 corresponds to a total absence of difference in color between healthy zones and zones with vitiligo, as can be evaluated by the human eye.

Statistical evaluation: the differences between the visits have been evaluated with the ANOVA test.

RESULTS

One patient dropped out from the study after the second visit due to lack of compliance in product application. Two patients did not come to the final visit (T6), for reasons inherent to their professional activity. No side effects were noted on the sites were the sunscreen was applied. The sunscreens did not have apparently any influence on disease activity but their use almost completely inhibited the appearance of repigmentation on the affected areas.

Sun exposure habits

The results of the questionnaire on sun exposure habits, that the patients had to fill at each visit, are summarized in table1. The survey demonstrated that all the patients were exposed daily for more than 1 hour to the sun and for a minimum of 10 days per month, during all the period of the study.
This questionnaire was aimed at determination of the daily mean exposure time during the period before the visit, expressed as sun exposure hours per day.

Table 1:
Characteristics of the patients and sun exposure habits.

Patient	Age	Sex	Skin type	disease activity	T2 sun exposure		T4 sun exposure		T6 sun exposure	
					hrs/day	Days	hrs/day	Days	hrs/day	Days
1	55	F	III	stable	3.4	13	1.5	10	1.3	12
2	38	F	III	active	4.2	16	2.7	17	1.6	15
3	52	F	III	active	3.1	12	4.3	21	2.2	13
4	55	F	IV	active	2.3	14	3.3	14	1.8	10
5	46	M	III	stable	3.5	13	-	-	-	-
6	49	F	III	stable	3.2	15	3.8	20	1.5	11
7	36	F	III	stable	2.4	10	4.6	21	1.3	12
8	34	F	III	active	1.3	14	3.9	17	1.4	10
9	31	F	III	active	2.5	13	3.6	19	1.7	15
10	30	F	III	stable	4.7	20	2.2	12	1.2	10
11	43	F	IV	stable	2.4	15	4.4	17	1.7	11
12	47	F	III	stable	1.8	10	3.8	24	1.9	12
13	29	F	III	stable	2.7	15	5.4	21	2.3	13
14	39	F	III	active	1.6	12	4.3	22	2.2	10
15	43	F	III	stable	2.2	15	4.1	25	1.3	11
16	49	F	III	stable	2.8	16	3.5	22	1.1	13
17	44	F	IV	stable	4.2	23	3.1	17	1.3	15
18	34	F	III	stable	2.7	21	3.3	18	-	-
19	53	F	III	stable	2.1	18	3.7	23	1.7	15
20	40	F	III	stable	2.5	16	3.2	14	-	-

On the basis of both the instrumental and clinical evaluation, in all patients there was an overall progressive decrease in the difference in pigmentation between healthy skin and vitiliginous skin, on the face and hands, where the sunscreen was applied. This difference became even more evident at T4 and T6 (fig 1a-1b, 2a-2b, 3a-3b). The difference was statistically more significant on the back of the hands as compared to the face. This finding may be due to the different exposure pattern of the hands as compared to the face.

Instrumental evaluation (skin reflectance)

- **Face**

As regards the face, in all patients there was an overall progressive decrease in the difference in pigmentation as measured with the derma spectrometer between healthy skin and vitiliginous skin. Table 2 shows the values of the MI on unaffected skin (US) and vitiliginous skin (V) as well as the differences between US and V, defined as ΔMI. The difference in MI at the various visits did not show statistically significant variations during the treatment.

Table 2:
Melanin index measured on the face at the various visits

T2		T4	
- Unaffected skin (US)	492 ± 13	- Unaffected skin (US)	491 ± 15
- Vitiliginous skin (V)	473 ± 10	- Vitiliginous skin (V)	473 ± 11
- ΔMI: US - V	19 ± 12	- ΔMI: US - V	17 ± 13

T6	
- Unaffected skin (US)	491 ± 16
- Vitiliginous skin (V)	472 ± 12
- ΔMI: US - V	20 ± 11

- **Hands**

On the contrary, as regards the hands there was a highly significant statistical difference in MI between T0 and T2. This difference became even more evident at T4 and T6.

Table 3:
MI measured on the hands at the various visits

T0		T2	
- Unaffected skin (US)	502 ± 15	- Unaffected skin (US)	503 ± 16
- Vitiliginous skin (V)	454 ± 9	- Vitiliginous skin (V)	452 ± 9
- ΔMI: US - V	48 ± 18	- ΔMI: US - V	51 ± 20

T4		T6	
- Unaffected skin (US)	501 ± 14	- Unaffected skin (US)	499 ± 11
- Vitiliginous skin (V)	452 ± 10	- Vitiliginous skin (V)	452 ± 11
- ΔMI: US - V	50 ± 15	- ΔMI: US - V	46 ± 10

- **Comparison of hands at visits T2, T4 and T6**

The MI was significantly reduced on hands but only on unaffected skin.
The ΔMI varied significantly at T4 and at T6.
The ANOVA for repeated measurements shows an interaction between visit and treatment.

Clinical evaluation

On the face no statistically significant differences in the clinical score have been noted between T0 and the following visits. On the contrary a highly significant statistical difference has been remarked on the hands: the score became progressively higher in the hands during the treatment period. This corresponds to a reduction in skin pigmentation on the healthy skin.

Fig. 1a-1b

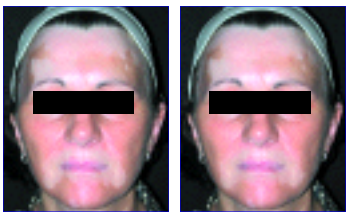


Fig. 2a-2b

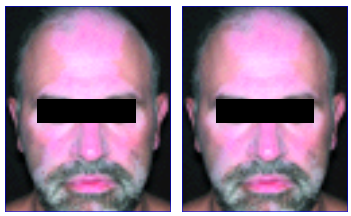
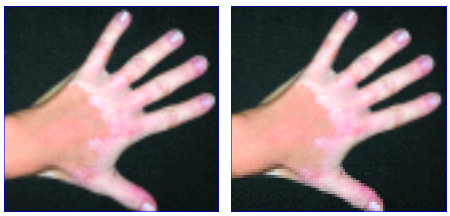


Fig. 3a-3b



DISCUSSION

We consider that the total duration of the study (6 months) and the fact that during these months the climate in central Italy allows prolonged sun exposure has permitted the exposure to a sufficient UV dose, that in the absence of adequate photoprotection can stimulate and maintain pigmentation. The questionnaire on solar exposure demonstrates that the majority of patients are exposed for longer times during the day and for a higher number of days per month during the central period of the study (evaluation at T4) that corresponds to the months of July and August (table 1). This is the period more at risk for vitiligo patients to develop antiaesthetic contrast between affected and normal skin. It must also be considered that in the Mediterranean countries it is not easy for the patients to strictly avoid sun exposure during the summer without compromising social life and consequently global quality of life. This seems to be particularly true mainly in young adult patients and the problem seems to affect more females than males.
In particular the results of skin reflectance measurements (statistical analysis) demonstrate that Anthelios XL is capable of offering adequate protection against hyperpigmentation of unaffected skin in vitiligo patients. The difference was noted mainly on the back of the hands and not on the face. Clinical evaluation confirmed, at least in part, the instrumental results.
There might be two possible explanations for this finding: the different pattern of solar exposure between the hands and the face (hands may be exposed for longer periods and receive UV rays perpendicularly), and the possibility that patients do not apply the same quantity of product on the hands and on the face for cosmetic reasons.
The ability of a sunscreen to inhibit pigmentation in normal skin is certainly related to its broad spectrum absorption, to the SPF and to its protection against UVA. To be effective, the products with high SPF need to have a corresponding high protection factor for UVA. These may be the reasons for which Anthelios XL has proved to be significantly effective.

CONCLUSIONS

The results of this clinical study indicate that Anthelios XL 60+ was very effective in preventing excessive tanning in patients affected with vitiligo that were exposed to a high level of solar radiation during the summer in the Mediterranean area. A higher efficacy was noticed on the hands of the patients as compared to the face.
On the basis of these results Anthelios XL 60 + can be recommended as a sunscreen of choice for patients with vitiligo that do not want to, or cannot, avoid solar exposure during summer in Mediterranean countries or other locations where solar irradiation is particularly intense.

LA ROCHE-POSAY
LABORATOIRE PHARMACEUTIQUE