

Photoprotection in Vitiligo and Normal Skin

A Quantitative Assessment of the Role of Stratum Corneum, Viable Epidermis and Pigmentation

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Pigmentation, stratum corneum and viable epidermis are considered to be the main factors protecting against ultraviolet radiation. We quantitatively investigated the degree of photoprotection provided by these structures in vitiligo and adjacent normally pigmented skin. In 14 patients 61 MED tests were performed in vitiligo and adjacent normally pigmented skin using a solar simulator. The thickness of stratum corneum and viable epidermis was determined from frozen skin sections, and pigmentation was calculated by measuring skin reflectance at 555 nm and 660 nm. To analyse photoprotection, the UV dose necessary to evoke erythema was regressed against the thickness of stratum corneum and viable epidermis, pigmentation and the erythema grade in the MED test. By analysing regression coefficients we found that stratum corneum was the main photoprotective factor not only in vitiligo but also in normally pigmented skin. The effect of pigmentation in normal skin was slightly less prominent. Stratum corneum was thicker in vitiligo than in normally pigmented skin. However, the photoprotection due to stratum corneum was similar in both groups because significantly less photoprotection was achieved per thickness unit of stratum corneum in vitiligo than in normal skin. Neither in vitiligo nor in normally pigmented skin did the photoprotection depend on viable epidermis.

Our data quantitatively document the importance of stratum corneum and pigmentation. Hyperkeratosis in vitiligo offers just as efficient photoprotection as does the normal stratum corneum in pigmented skin. **Key words:** ultraviolet radiation; erythema.

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Exposure to ultraviolet radiation exerts various deleterious effects in the skin including inflammation, immunosuppression, photodamage and carcinogenesis. A number of protective mechanisms have been developed during evolution to protect human skin from excessive UV radiation. There is evidence that stratum corneum protects against UV mainly due to reflection of radiation, absorption of the radiant energy and internal scattering (1). In the palms where stratum corneum is very thick (500 µm) the minimal erythema dose is 16 times higher than on the back, where stratum corneum is only 19 µm thick. It was observed that in areas stripped from stratum corneum, sunburn develops more easily than in the unstripped areas of the skin (1).

Melanin is a potent UV absorber, and thus skin pigmentation is another natural protective factor against UV radiation. It is well known that pigmented skin is more resistant to sunburn than poorly pigmented skin (2, 3). Delayed effects of chronic UV exposure, such as development of skin cancer and

photoageing, are also more pronounced in individuals with fair skin colour (4–7).

Despite the well recognized photoprotective role of the stratum corneum and skin pigmentation, quantitative data on the modulation of photoprotection by these factors are missing. The role of viable epidermis in sun-protection also remains to be elucidated.

Vitiligo provides an interesting model for investigating skin photoprotection. Skin in vitiligo does not contain pigment and thus the protection against UV is afforded by the stratum corneum and the rest of epidermis. It might thus be suspected that hyperkeratosis and epidermal hyperplasia would develop in vitiligo exposed to UV to compensate for the lack of pigment.

Here, we determined the photoprotective capacity of stratum corneum, viable epidermis and pigmentation in normally pigmented skin and compared the photoprotective capacity of these factors in vitiligo with normally pigmented skin.

MATERIAL AND METHODS

Patients and experimental procedures

Fourteen patients with vitiligo (3 men, 11 women, median age 31 years, range 18–63 years) entered the study upon informed consent. Experiments were performed in November and December. The subjects had not received light therapy or been exposed to the sun for 3 months before enrolment in the study.

For determination of thickness of stratum corneum and viable epidermis, 3 mm superficial excision biopsies were taken under a local lidocaine anaesthetic from 61 areas of vitiligo and adjacent normally pigmented skin. Regions habitually exposed to the sun (dorsal forearm, dorsal hand, dorsal foot, anterior lower limb, upper anterior chest) and sun-protected regions (volar forearm, buttock) were examined. Biopsies were frozen in isopentane (2-methylbutane) at –80 °C and stored at that temperature. Thickness of stratum corneum and viable epidermis was measured in frozen sections in a light microscope. Seven measurements were performed on each section, and the mean value was used for further analysis.

To determine the sensitivity of the skin to UV (MED), we assessed the skin erythema reaction 24 h after irradiation from a xenon solar simulator (Model 601, Solar Light, USA). The test sites within vitiligo and normal skin were irradiated with the xenon solar simulator using 25% dose increments in 6 circular sites, each of 0.9 cm in diameter.

Erythema in the irradiation sites was scored on a 5-point visual scale: 0: no difference from surrounding skin; (+): diffuse mild erythema with badly defined borders; +: uniform erythema with sharply defined borders; ++: bright red colour with slight induration (oedema) on palpation; and +++: heavy bright red colour and pronounced induration (oedema) above surrounding skin.

To establish the appropriate range of doses for a given individual, a "UV-Optimize, 555" equipment (Matic, Denmark) was used, as described previously (8–10). Briefly, the instrument measures skin reflectance at 555 nm and 660 nm, which makes it possible to quantify skin redness (%) and pigmentation (%) independently. These values allow for the calculation of a pigment protection factor (PPF) and

prediction of the UV dose necessary for the induction of erythema. PPF expresses the number of SED (standard erythema dose = 31.2 J/cm²/296 nm) necessary to provoke a + erythema reaction in a group of healthy very sensitive Caucasians.

Data analysis

Mean values \pm standard error of the mean (SEM) are presented. A single or multiple linear regression analysis was used to determine the contribution of the explanatory variables (thickness of stratum corneum, viable epidermis and pigmentation) to the dose of UV necessary to evoke an erythema reaction in the skin. The increase in the UV dose was interpreted as an increase in skin photoprotection capacity.

A multiple linear regression model for the normally pigmented skin was expressed as:

$$\text{UV dose [SED]} = a \times \text{thickness of stratum corneum } [\mu\text{m}] \\ + b \times \text{thickness of viable epidermis } [\mu\text{m}] \\ + c \times \text{pigmentation } [\%] + d \times \text{erythema grade} + e. \quad (1)$$

The erythema grade is an ordered categorical variable, and for the purpose of analysis it was coded as 0.5 for (+) reaction, 1 for + reaction, 2 for ++ reaction and 3 for +++ reaction. The *a-d* represent regression coefficients of the explanatory variables and the *e* is a constant. The linear association between the dependent variable and the explanatory variables was documented by the absence of curvature in the plots of the residuals against the variables (11). Independence of explanatory variables is assumed for the multiple regression model. Since no apparent relationship exists between pigmentation and the thickness of epidermis and stratum corneum (3, 12), the only biologically sound interaction could be that of the thickness of stratum corneum and viable epidermis. This was examined by creating a new variable, which was their product, and adding it to the model. Since the new model did not show an improved fit (tested via the F statistics), it was assumed that the effects of the thickness of the epidermis and the thickness of the stratum corneum were independent (11).

For vitiligo data a similar multiple regression model was employed, where the product "*c* \times pigmentation" was assumed to equal zero.

Regression coefficients are interpreted as the estimated increase in the outcome variable for an increase of one unit in the predictor variable (11). Thus, the average photoprotection afforded by stratum corneum and pigmentation was calculated by multiplying relevant regression coefficients by average thickness of stratum corneum or pigmentation.

To investigate if the thickness of stratum corneum and viable epidermis differed from that of normal skin, we expressed stratum corneum and epidermal thickness as an index to eliminate the region-dependent variability:

$$\text{Index of thickness} = (\text{thickness, vitiligo}) / \\ (\text{thickness, adjacent pigmented control skin}) \quad (2)$$

The value of 1 means identical thickness of viable epidermis or stratum corneum in the region of vitiligo and the adjacent pigmented skin.

The statistical evaluation of the index was performed with the single sample *t*-test and the between-group comparisons were made with a two-sample *t*-test. *p* < 0.05 was considered significant.

RESULTS

Photoprotection in normally pigmented skin

The relationship between thickness of stratum corneum and UV diagnosis required to provoke a given erythema grade is illustrated in Fig. 1.

The multiple linear regression analysis yielded a model presented in equation (1) and Table I. Tests of the hypothesis that individual regression coefficients equalled zero were per-

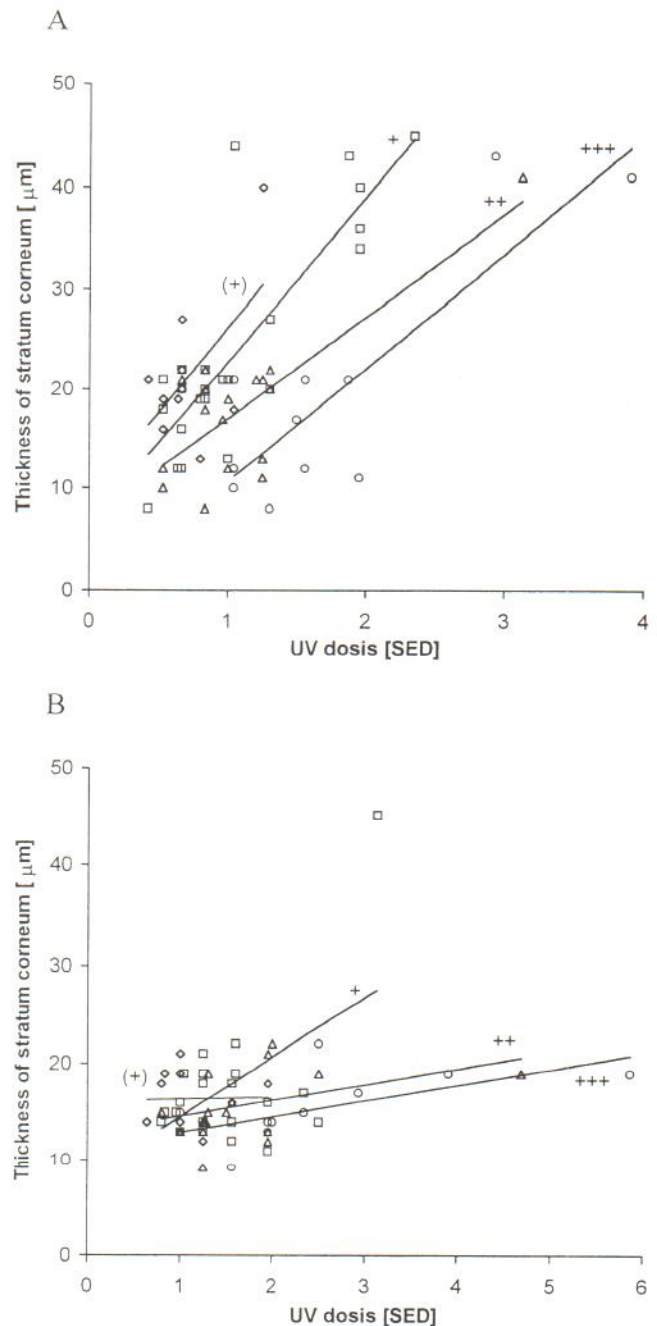


Fig. 1. Relationship between the thickness of stratum corneum and the dose of UV required to provoke skin erythema in vitiligo (A) and normally pigmented skin (B). The dose is expressed in SED; erythema was scored on an ordinal scale as described in *Material and Methods*. The minimal UV doses necessary to provoke an erythema of a particular grade were plotted against the stratum corneum thickness. \diamond , (+); \square , +; \triangle , ++; \circ , +++. For each grade of erythema, regression lines were fitted by simple linear regression using the thickness of stratum corneum as a predictor variable.

formed, and *p*-values were calculated for each coefficient. Statistically significant regression coefficients were obtained for the thickness of stratum corneum and pigmentation, confirming their role in photoprotection. The regression coefficient for viable epidermis was not significant. Regression coefficients are interpreted as the estimated increase in the outcome

Table I. Role of the thickness of stratum corneum, pigmentation and viable epidermis in photoprotection quantified from the multiple regression model (equation (1))

Regression coefficients (mean \pm SEM), with corresponding *p*-values are shown for normally pigmented skin and vitiligo.

Explanatory variable	Regression coefficient	
	Pigmented skin	Vitiligo
Stratum corneum	0.064 \pm 0.019 <i>p</i> = 0.002	0.050 \pm 0.005 <i>p</i> < 0.0001
Pigmentation	0.037 \pm 0.014 <i>p</i> = 0.008	–
Viable epidermis	–0.0005 \pm 0.006 NS	–0.003 \pm 0.002 NS

variable for an increase of one unit in the predictor variable (11). Therefore we could predict the average photoprotection afforded by the stratum corneum and the pigmentation, respectively, in our experimental group. Thickness of stratum corneum was 16.5 \pm 0.6 μ m and mean pigmentation was 21.3 \pm 1%. The average photoprotection provided by the stratum corneum was 1.06 \pm 0.32 SED (calculated from equation (1): 0.064 \pm 0.019 SED/ μ m \times 16.56 \pm 0.6 μ m). The corresponding value for pigmentation was 0.80 \pm 0.29 SED (0.037 \pm 0.014 SED/% \times 21.3 \pm 1%). Thus, in our experimental group the photoprotection afforded by the stratum corneum was approximately 32% higher than that of pigmentation and was estimated to be 57% of the total protection.

Photoprotection in vitiligo

In vitiligo, the stratum corneum was thicker than in the adjacent pigmented skin, both in the areas of habitual high- and low-exposure to UV (Table II). No differences were detected in the indexes of thickness for stratum corneum and viable epidermis between high-exposed and low-exposed regions.

The multiple regression analysis for vitiligo yielded a statistically significant regression coefficient for the thickness of the stratum corneum and an insignificant coefficient for the thickness of viable epidermis (Table I). Interestingly, the regression coefficient for the thickness of stratum corneum in vitiligo

Table II. Thickness of the stratum corneum and viable epidermis (mean \pm SEM) in vitiligo given as indexes calculated by dividing the values for vitiligo by those of the control adjacent pigmented skin (see equation (2))

The difference in indexes of thickness between high-exposed and low-exposed sites was insignificant.

	Index of thickness ^a	
	Stratum corneum	Viable epidermis
High-exposed sites	1.45 \pm 0.86 ^a	1.10 \pm 0.33
Low-exposed sites	1.30 \pm 0.35 ^a	1.14 \pm 0.22 ^a
All sites	1.36 \pm 0.63 ^a	1.12 \pm 0.27 ^a

^a*p* < 0.05, single sample *t*-test versus 1.

differed significantly from that obtained for normally pigmented skin (*p* < 0.001, two-sample *t*-test). This suggests a qualitatively inferior photoprotective capacity of stratum corneum in vitiligo, since greater photoprotection is achieved for 1 μ m of stratum corneum in normally pigmented skin than in vitiligo. This is further illustrated in Fig. 1, where it is shown that individual regression lines for different grades of erythema have lower slopes for normally pigmented skin than for vitiligo. Using the cumulative regression coefficients for stratum corneum obtained from the multiple regression model for vitiligo (Table I) and multiplying them by the corresponding values of mean thickness of stratum corneum, an average photoprotection provided by stratum corneum is predicted to equal 1.06 \pm 0.12 [SED] (0.05 \pm 0.005 SED/ μ m \times 21.2 \pm 1.3 μ m). Thus, the average photoprotection provided by hyperkeratotic stratum corneum is not significantly different from that of normal stratum corneum.

DISCUSSION

The results of the study document that interindividual variability in pigmentation or thickness of stratum corneum significantly affects the photoprotective capacity of the skin. The multiple regression model applied here showed that in normally pigmented skin of our study group the photoprotection provided by stratum corneum was 32% higher than that due to pigmentation. This result supports earlier observations *in vitro*, where transmission of UV through stratum corneum and epidermis was measured separately. It has been reported that 48% of solar simulated light is transmitted through Caucasian stratum corneum and 29% through whole pigmented epidermis (3). If we disregard an effect of viable epidermis we may infer that UV protection of stratum corneum is approximately 39% higher than that of pigmentation, a result which falls within the ranges obtained in the present study.

The photoprotective role of pigmentation has not been definitely elucidated. Some authors have considered it to be the major UV protectant (1, 2, 8, 12), whereas others underlined that melanin in Caucasians is a relatively weak sunscreen (13–15). Present data support the latter view and stress the dominant sunscreen role of stratum corneum in Caucasians. However, the photoprotective role of melanin must not be disregarded; pigmentation is clinically significant and it is well established that individuals with fair skin complexion readily develop erythema and are prone to the development of skin cancer (4–7). This stresses only the role of stratum corneum, which should be taken into account in predicting the effect of UV on skin.

In view of the demonstrated negligible role of viable epidermis in photoprotection, stratum corneum is probably a major UV protective factor in vitiligo. We found an increased thickness of the stratum corneum in vitiligo, both in habitually sun-exposed and low-exposed regions of skin. Increased thickness of stratum corneum is known to develop upon exposure to UV, and this phenomenon is related to the protection against UV radiation. In this regard, a similar degree of thickening of the stratum corneum in high- and low-exposed skin was unexpected. We expected a more pronounced change in exposed skin, since UV is directly responsible for epidermal hyperplasia, acceleration of keratinocyte differentiation and thickening of the stratum corneum a short time after exposure. Our experiments were performed during the winter months,

when the degree of UV radiation in Denmark is very low and "sun-protection" with clothes takes place even in sites classified as high-exposed. We have therefore practically eliminated acute effects of sun exposure being left with chronic changes only. Under such conditions stratum corneum is still thicker in vitiligo than normally pigmented skin, and no differences between high-exposed and low-exposed regions were detected.

The differences in the thickness of stratum corneum between normal skin and vitiligo have, to our knowledge, not been reported before. In a single report, Everett (16) showed that after UV irradiation with a mercury lamp, the thickness of the stratum corneum increased in vitiligo, but not in control pigmented skin. The author also presented results on the measurements of the thickness of stratum corneum in non-irradiated regions, but because of a low number of investigated persons no statistically meaningful conclusions could be drawn.

Comparison of regression coefficients led to the conclusion that the photoprotective capacity of stratum corneum in vitiligo per unit of thickness was inferior to that in normal skin. Therefore, in spite of hyperkeratosis in vitiligo the average photoprotection provided by stratum corneum was unchanged. The reason for a lower photoprotective capacity per unit of stratum corneum in vitiligo remains speculative. Differences in pigmentation of stratum corneum are unlikely to be an explanation, since the effects of this variable were removed with the "pigmentation" variable in the multiple regression model. It is possible that the structure of stratum corneum (the number of layers, compactness) is different. The coherence of stratum corneum determines the quality of the skin barrier (17), and thus it is also likely to influence photoprotection.

The epidermis has been considered to act as an optical barrier by absorbing and scattering UV. Studies in vitro revealed that UV absorption by epidermis obeys the Lambert-Beer law, which states that transmission decreases proportionally with increasing thickness (18). However, in our experimental group photoprotection did not depend on the viable epidermis since the regression coefficients for viable epidermal thickness did not attain statistical significance either in vitiligo or in normal skin.

One of the reasons why epidermis is ineffective as a UV-screen in vivo might be that the optic characteristics of epidermis in vivo differ from those observed in vitro. If epidermal photoprotection depends on scattering rather than UV absorption, the Lambert-Beer law is not applicable and consequently no relationship between UV protection and epidermal thickness may be observed. Alternatively, it may be argued that, when compared with stratum corneum, the epidermal concentration of biological materials which absorb UV is relatively low due to a high water content. Experimental data which show that UV sensitivity of the skin may be elevated by increasing the degree of epidermal hydration (19, 20) are in accordance with this hypothesis.

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