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ORIGINAL ARTICLE

A broad spectrum high-SPF photostable sunscreen with a high UVA-PF can protect against cellular damage at high UV exposure doses

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SUMMARY

Background
Advances in sunscreen technologies have yielded broad spectrum sunscreens at high-sun protection factor (SPF) and ultraviolet A protection factor (UVA-PF) levels that are photostable and powerful in protecting skin from erythema. Questions arise whether these sunscreens protect proportionally against cellular skin damage caused by high ultraviolet exposures.

Objective
The objective of this study is to evaluate if high-SPF sunscreen can protect skin at a cellular level under UV exposure doses [> 50 minimal erythema dose (MED)] similarly to the SPF value.

Methods
Sunburn cells, Langerhans cells, thymine dimers, protein 53 (p53), and matrix metalloproteinase (MMP)-1 and MMP-9 endpoints were evaluated in biopsies from 12 subjects following four treatments: unprotected exposed to 0, 1 and 3 MED and sunscreen (SPF 55) protected exposed to 55 MED of UV radiation.

Results
All the markers showed significantly more damage for the 3 MED-untreated sites when compared with non-irradiated control, and majority of the markers showed marked damage following unprotected 1 MED exposure. After 55 MEDs, sunscreen-protected sites showed significantly less p53 and MMP-9 (keratinocyte) staining than the 1 MED-exposed unprotected sites, while all the other biomarkers in sunscreen protected sites showed no statistical differences from 1 MED-exposed unprotected sites.

Conclusions
A high-SPF photostable sunscreen with high UVA-PF can provide proportionately high protection against multiple cellular damage markers.

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Sunscreens can play an important role in protecting skin from damaging ultraviolet radiation (UVR) as part of an overall sun avoidance strategy that includes use of protective clothing, seeking shade and avoidance of direct exposures during the times of the peak sun hours. The magnitude of sunburn protection, or sun protection factor (SPF), is determined based on the clinical observation of the protection against acute 'minimal erythema' response in human skin under controlled laboratory conditions as prescribed by the FDA Monograph. 'Minimal erythema' response in skin is very well documented (1), and skin redness is a direct and an easily visible marker of the damage. The use of sunscreen to protect against erythema reaction or sunburn is also very relevant to consumers, especially to people with light or fair skin color.

However, 'minimal erythema' is just one of the clinical endpoints to evaluate UVR-induced skin damage. SPF testing of sunscreens establishes the level of protection against a visible 'sunburn' response but does not necessarily guarantee the same level of protection against other cellular damage which may have different action spectral profiles or different dose–response relationships relative to erythema. Changes at a cellular or molecular level can be measured at UVR doses far below those needed to elicit a minimal erythema response (2). Simply because the change is not visible to the naked eye, or any other sensory cue, does not mean that it is unimportant. Numerous studies (3–7) have shown that sub-erythemal UVR doses can cause long term and significant damage to tissue, resulting in both cosmetic defects (wrinkles, sagging, dark pigment spots) as well as pathological damage (immune suppression and skin cancer formation).

In the past, sunscreens filtered primarily UVB, with much lower levels of UVA protection as a result of limited number of approved UVA absorbers and/or limited photostability of the best UVA absorber (i.e. avobenzone) (8). With the development of photostabilizing technologies and the advance in formulation technologies, modern sunscreens can provide photostable, broad spectrum protection with both high SPF and UVA protection factors (UVA-PFs). New concern has been raised that the use of these very high SPF sunscreens may efficiently prevent the visible sunburn reaction even under high UVR exposure doses to the extent that cellular damage could accumulate without being visibly noticed. Recently, a concern has been expressed that sunscreen filters may be acting as 'anti-inflammatory' agents (9), diminishing the visual erythema effect (and inflating the SPF value in clinical testing) while masking invisible cellular and structural damage. This study was designed to evaluate whether the level of protection provided by a high SPF broad spectrum photostable sunscreen with a high UVA-PF indeed provided protection against UVR damage at a cellular and molecular level equivalent to that of the erythema protection (SPF). We evaluated and documented for the first time the protective effects against cellular skin damage for high SPF (SPF > 50) broad spectrum sunscreen following high solar simulated UV exposure [> 50 minimal erythema dose (MED)].

MATERIALS AND METHODS

Test material

A SPF 55 broad spectrum sunscreen lotion containing 10% homosalate, 5% oxybenzone, 2.8% octocrylene, 5% octisalate and 3% avobenzone was evaluated in this test. This product was determined to have a UVA-PF of 22 by the persistent pigment darkening protocol *in vivo* (10). It was also shown to be highly photostable after 50 MED by an *in vitro* method (8), and the critical wavelength of this product was determined to be 376 nm according to the protocol outlined by FDA in 2011 (11). The sunscreen also meets the European Union and Australian UVA requirement for the ratio of SPF to UVA-PF to be less than 3.0.

Subjects

The study protocol and the informed consent conforming to 21 CFR 50.25 and HIPAA were reviewed and approved by an Institute Review Board prior to the study. Twelve subjects (nine female, three male), ranging in age from 33 to 59 and Fitzpatrick skin type I–III (four subjects for each skin type), were enrolled into the study. The study was conducted at a clinical facility in Texas in March.

Determination of Minimal Erythema Dose

A phototest was conducted on the lower back of each subject to determine their individual MED. A xenon arc solar simulator (Solar Light Model 12S, Philadelphia, PA, USA) compliant to the COLIPA (12) and FDA (11) specifications was used throughout this study. A series of seven irradiation exposures increasing in 25% increments was used to determine the MED. Twenty-four to twenty-eight hours after the completion of the irradiation, the irradiated sites were examined for erythema. The irradiation site that received the lowest dose of UVR which caused a perceptible redness with clear borders was chosen as the MED for the subject.

Test treatments

After MED determination, four treatments were conducted on the lower back of each test subject:

• Untreated control: No topical treatment with no UVR exposure.
• Positive control 1: No topical treatment with 1 MED UVR exposure.
• Positive control 2: No topical treatment with 3 MEDs UVR exposure.
• Test treatment: Topical SPF 55 treatment with 55 MEDs UVR exposure.

The sunscreen was applied at the standard application dose of 2 mg/cm^2 and allowed to dry for a minimum of 15 min prior to UV exposure. The test sites were then irradiated with the solar-simulated UVR according to the study design above. Erythema and immediate pigment darkening (IPD) were graded immediately upon completion of each UVR exposure.

Twenty-four to twenty-eight hours after the end of the last UVR exposure, the treated sites were evaluated for visible responses for erythema. The erythema grading was the following: 0 = no erythema; 0.5 = unclear redness; 1 = eyrthema with boarders; and 2 = clear erythema with or without edema. Two-millimetre circular punch biopsies were collected with standard procedures from each of the four treatment sites. The tissue samples were fixed in 10% buffered formalin and send to ProPath Laboratories (Dallas, TX, USA) for processing and immune staining of the tissues.

Histological evaluations

Tissues were prepared and stained to evaluate the presence of Langerhans cells, matrix metalloproteinases (MMPs), sunburn cells, thymine dimer (TT) and protein 53 (p53), a transcription factor that regulates cell cycle and suppresses tumour growth. Digital photomicrographs were taken and were evaluated for each of these endpoints by a board-certified dermopathologist who was blinded to the treatments. For Langerhans cells (CD1a staining), the number of cells exhibiting moderate to strong positive staining cytoplasm and a visible nucleus was counted per millimetre of epidermis. For sunburn cells (haematoxylin-eosin staining), the number of keratinocytes with dense hypereosinophilic cytoplasm and dark small picnotic nuclei was counted per millimetre of epidermis. For thymine dimer immuno-histochemical staining, the number of nuclei that showed positive staining per millimetre of the epidermis was counted. For immune-histochemical staining of MMP-9, a 5-point scale (0–4) was used to grade the level of the staining. For p53, the number of cells exhibiting strong positive staining (grade 3 and 4 in a 5-point grading scale of 0–4) per millimetre of the epidermis was counted. Results for each of the endpoint and each of the treatment were averaged for all the subjects, and the four treatments were compared by analysis of variance (ANOVA) followed by paired comparisons (Fisher's LSD test) with significance level of 0.05.

RESULTS

The untreated and un-irradiated site had no pigment darkening and erythema either immediately or 24 hours following the treatment and served as the negative control of the study. The 3 MEDs exposed untreated sites served as the positive control in the study. Seven (7) subjects showed IPD and five (5) subjects showed immediate erythema following 3 MED of UVR exposure. Clear erythema (erythema grade = 2) was also observed for every subjects after 24 h for the 3 MED exposure.

The untreated 1 MED-exposed sites showed IPD for nine (9) out of the 12 subjects, while the sunscreen-treated sites (the 55 MED sites) showed IPD for all of the 12 subjects. No immediate erythema was observed for the 1 MED or 55 MED sites. After 24 h, the 1 MED-exposed sites showed erythema grade of 1 for every subjects, while the 55 MED sites showed erythema grade of 1 for seven subjects and grade of 0.5 for five subjects.

The immuno-histological evaluation was conducted on all 12 subjects ($N = 12$). Table 1 lists the mean and standard deviation for each of the cellular or molecular endpoint evaluated including the presence of CD1a-positive Langerhans cells, MMP-1 and MMP-9 in either keratinocytes or underlying stroma, sunburn cells, thymine dimers, and p53. Figure 1 compares the four different sites (sunscreen-protected site vs. 0, 1 and 3 MED-exposed sites) for each of the endpoint, also showing the grouping of different treatments based on the statistical analysis. The unexposed sites (negative control) were always statistically different from the 3 MED-treated unprotected sites (positive control) for each of these biomarkers, demonstrating the validity of the evaluations and the involvement of these biomarkers in UVR-induced skin damage. For thymine dimer, p53 and MMP-9, the 1 MED-exposed sites also showed statistical difference from the untreated negative controls, consistent with the sensitivity threshold reported previously (13, 14). The Langerhans cells and the MMP-1 in both keratinocytes and in stroma did not show statistically significant differences between 1 MED exposure and non-irradiated control, probably due to the large variations that existed among different individuals and the small sample size. Even though not statistically significant, the count of sunburn cells increased for 11 out of 12 subjects following 1 MED UVR exposure.

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Table 1. Comparison of means and standard deviations (± STD) for four different treatments: untreated and un-irradiated, untreated and irradiated with 1 or 3 MED, sunscreen treated (SPF 55) and irradiated with 55 MED ($N = 11$)

	Sunscreen treated, 55 MED irradiated	Untreated, un-irradiated	Untreated, 1.0 MED irradiated	Untreated, 3.0 MED irradiated
Langerhan's cells (CD1a+)	19.73	23.17	21.83	9.25
± STD	3.55	6.97	9.60	2.49
Sunburn cells	1.91	0.00	2.92	18.00
± STD	2.39	0.00	4.50	10.04
Thymine dimers	15.09	0.00	20.58	107.60
± STD	15.21	0.00	18.88	20.97
p53	27.91	2.50	56.42	43.08
± STD	21.35	1.24	21.60	19.34
MMP-1 within keratinocytes	0.27	0.08	0.33	0.58
± STD	0.65	0.29	0.49	0.51
MMP-1 within underlying stroma	0.36	0.08	0.08	0.42
± STD	0.50	0.29	0.29	0.51
MMP-9 within keratinocytes	0.45	0.25	0.83	1.67
± STD	0.52	0.45	0.39	0.65
MMP-9 within underlying stroma	0.73	0.00	0.92	2.00
± STD	0.90	0.00	0.79	0.43

CD1a, cluster of differentiation 1a; MED, minimal erythema dose; MMP, matrix metalloproteinase; p53, protein 53; SPF, sun protection factor.

For Langerhans cells, sunburn cells, thymine dimer MMP-1 in both keratinocytes and stroma, and MMP-9 in underlying stroma, the sunscreen-protected sites (55 MED) were not statistically different from 1 MED-exposed sites. These observations offer direct and clear evidence that a photostable, broad spectrum high-SPF sunscreen protected against damage at cellular and molecular levels even under this large UV exposure, confirming the significant protection was present even for these sensitive biomarkers and that the protection was at least as good as the protection of 'minimal erythema' reaction. For p53 and MMP-9 in keratinocytes, the sunscreen-protected sites (55 MED) showed statistically less damage than the 1 MED-exposed sites, suggesting that the sunscreen treatment may offer better protection to the underlying cellular damage than its SPF value. Figure 2a–e presented examples of the histological staining of the sunburn cells, Langerhans cells, TT, p53, and MMP-1 and MMP-9.

DISCUSSIONS

This study utilized a laboratory 'solar simulator' used for clinical sunscreen SPF determinations with tightly specified spectral characteristics. While mimicking solar radiation spectral qualities as closely as possible with existing filtration technologies, it should be noted that clinical solar simulators contain less long wavelength UVA-1 radiation (380–400 nm) relative to the solar UV spectrum. While unavoidable for clinical studies such as this, it represents a caveat to the observations and conclusions of this study.

The biomarkers from UVR exposure that had been widely studied for acute UV damage include the accumulation of thymine dimers (TT), the induction of p53 repair response (14–16), the increased expression of MMPs (17), the presence of sunburn cells and the disappearance of the Langerhans cells (18). DNA damage in epidermal cells is the primary and one of the most direct consequences of acute UV exposure. DNA can absorb UVB leading to the formation of thymine dimers (TT), which have been linked to the increased risk of developing skin cancer. One cellular response to DNA damage is apoptosis with formation of sunburn cells, and another is the increased level of p53 in keratinocytes because p53 is believed to be actively involved in the repair of DNA damage. Loss of Langerhans cells in epidermis can lead to photo-immunosuppression. Finally, the elevated expression of MMPs following UVR exposure plays a significant role in damaging extracellular matrix structures in dermis, contributing to premature signs of aging such as wrinkles and sagging (2, 15). We showed clear evidence in this study that high SPF sunscreen protects the cellular and molecular damage in skin. When compared with 1 MED-exposed sites, the sunscreen-protected sites showed no increase of damage

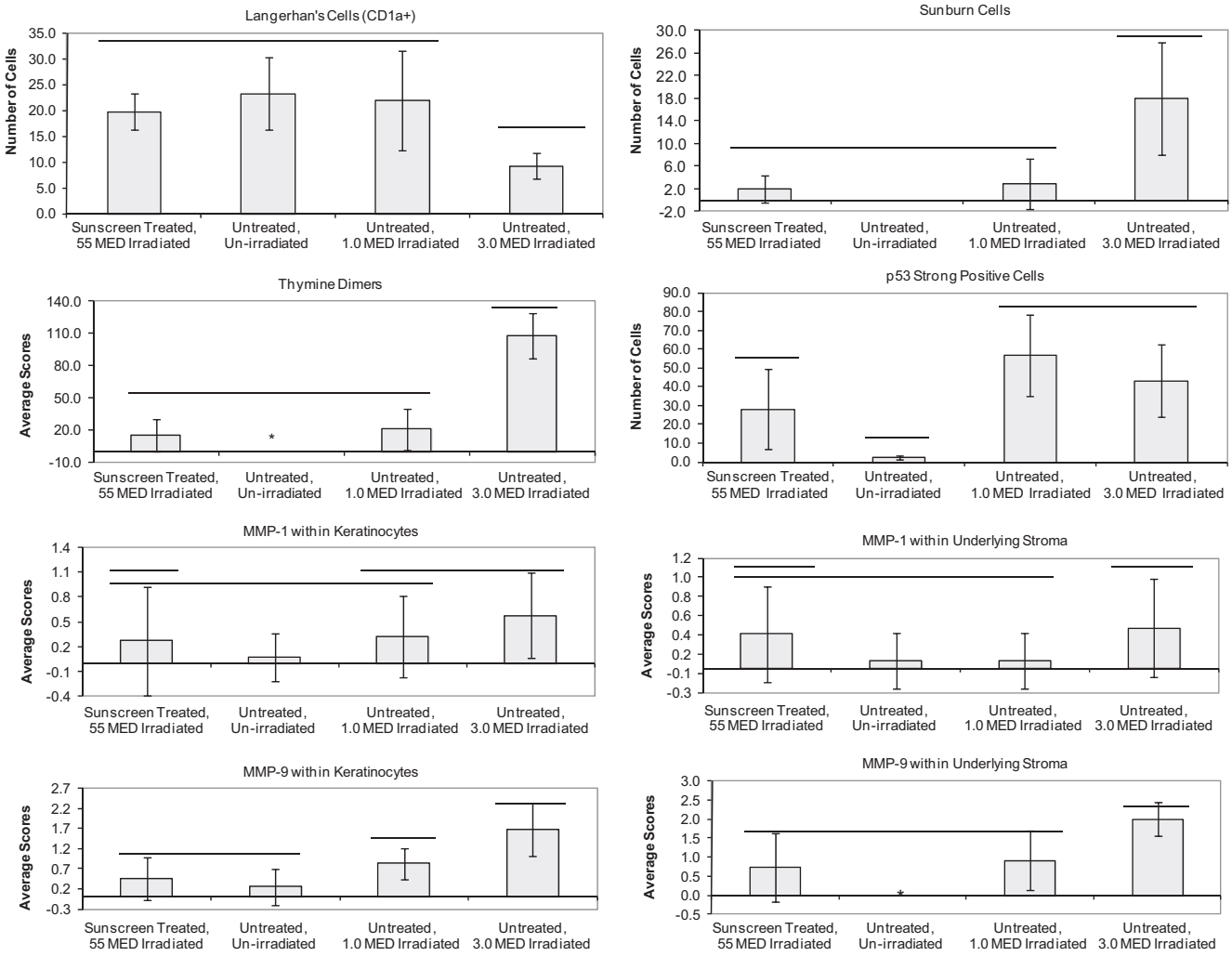

Fig. 1. Comparison of the effects of four treatments: untreated and un-irradiated, untreated and irradiated with 1 or 3 minimal erythema dose (MED), sunscreen treated (SPF55) and irradiated with 55 MED on Langerhans cells, sunburn cells, thymine dimer (TT), p53, matrix metalloproteinase (MMP)-1 and MMP9 in keratinocytes, and underlying stoma. Different groups as determined by statistical analysis are indicated by the horizontal bar. *Indicates those scores are zero.

for all the biomarkers tested when exposed to even 55 MED.

Several previous studies had shown that SPF 15 sunscreens were able to protect against currently tested biomarkers following acute UVR exposure of 2 MED (5, 14–16). However, daily UVR exposure for a normal person can easily pass 2 MED and can reach as high as 50 MED (11). We showed in this study that when applied sufficiently, the high SPF modern sunscreen can offer powerful protection against cellular and molecular damage in skin at UVR dose comparable with the SPF label. Even though the level of 50 MED UVR exposure is not common for many consumers, traditional SPF 15 sunscreen may not be sufficient for extended outdoor activities in the summer for a person with light skin. Modern high SPF sunscreen can also provide a margin of safety even when consumers under-apply the products and do not receive the protection level indicated by the SPF label (19).

These data also are relevant to the concerns that sunscreen agents (UV filters) are acting as 'anti-inflammatories' diminishing the erythema signal that is used to establish the SPF of sunscreen products in clinical testing and allowing more invisible cellular and structural protein damages to occur while the sunscreen users continue to sunbathe without developing a sunburn. None of the markers evaluated in this study showed damage levels above the expected 1 MED response. Had the 'anti-inflammatory' hypothesis been true, we would have expected to see responses significantly higher than the

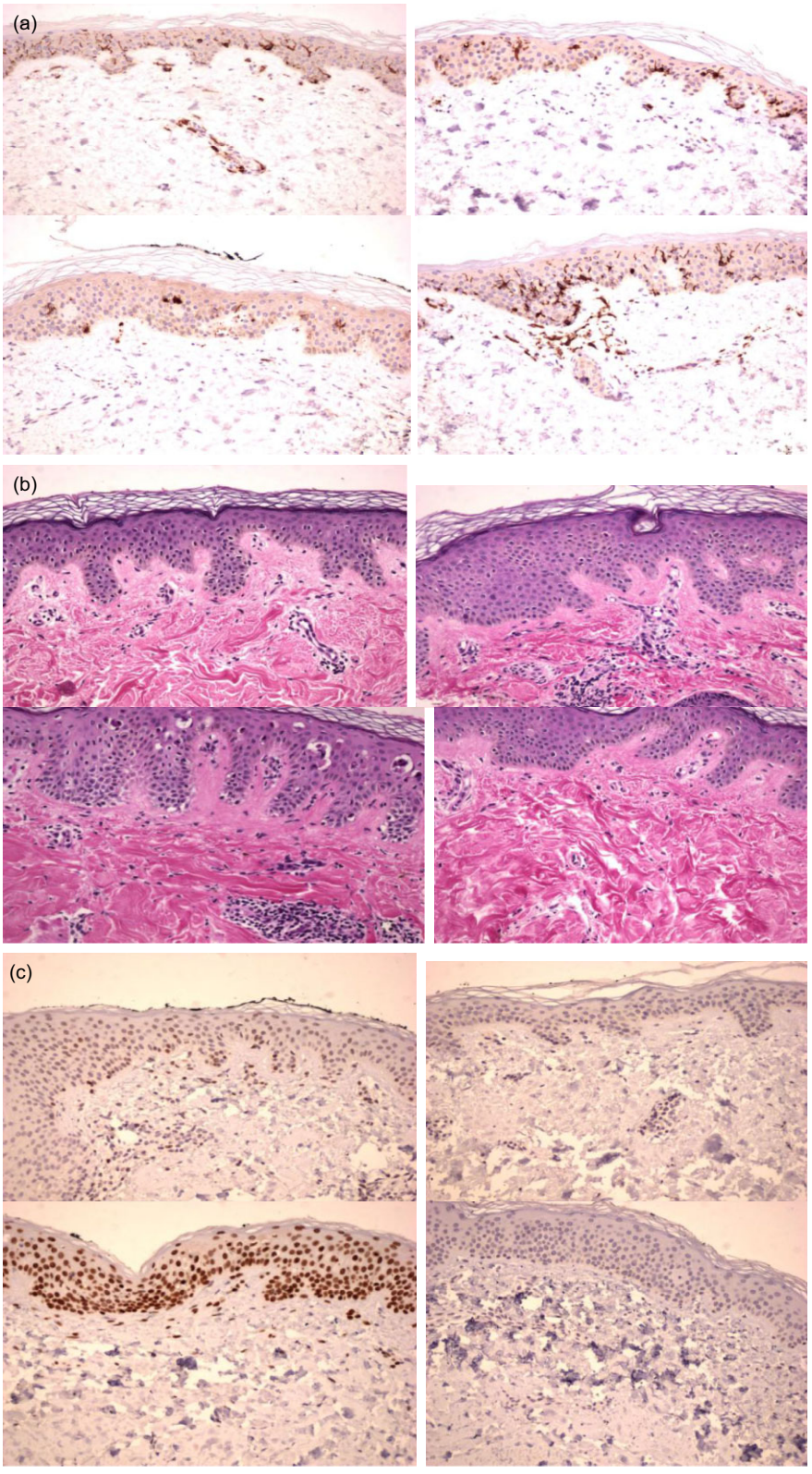

Fig. 2. Examples of histology staining from human skin biopsies for different biological endpoints. The treatments are 0 minimal erythema dose (MED) (top left), 1 MED (top right), 3 MED (bottom left) and 55 MED + sunscreen protected (bottom right). (a) Langerhans cells. (b) Sunburn cells. (c) Thymine dimers. (d) p53. (e) MMP-9.

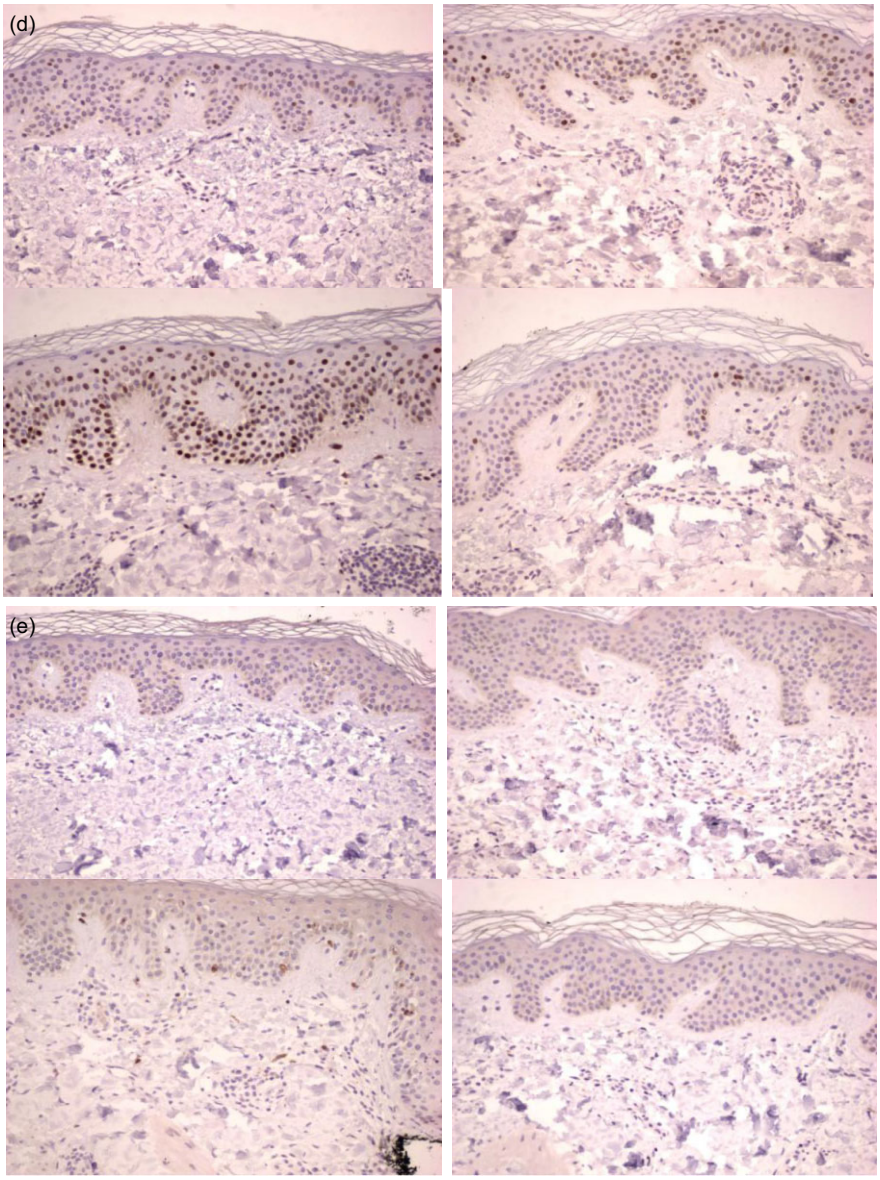

Fig. 2. *Continued*

unprotected 1 MED response level exposure and more similar to the unprotected 3 MED level responses.

There are no sunscreens that completely protect skin from solar UV exposure, and they should only be used as part of the overall sun avoidance practice. However, a high UVA-PF, broad spectrum high-SPF photostable sunscreen such as the one tested here was shown to offer additional cellular and molecular protection to skin that was proportional to the SPF level of the products. Repetitive sub-erythemal doses of UVR exposure had been shown to be able to generate significant cumulative biological damage without any signs of 'minimal erythema' (6, 7, 20). Our data show that the sunscreen tested in this study also protected at the cellular level, minimizing the underlying biological toll to levels equivalent to or significantly better protection (e.g. p53 and MMP-9) against equivalent sunburn response. This suggests that protection from this type of sunscreen (broad spectrum, high SPF, high UVA-PF, photostable) would not exacerbate sub-erythemal cellular damage upon cumulative exposure more than unprotected skin at equivalent sunburn response levels.

In conclusion, we demonstrated in this study that a broad spectrum high-SPF photostable sunscreen with high UVA-PF (SPF/UVA-PFA ratio < 3) such as the one tested in this study can protect against multiple damage biomarkers at the cellular level in skin even under high UVR exposure with efficacy similar to the SPF number. We found no evidence that sunburn protection offered by high

SPF sunscreen masks any 'invisible' cellular damage in skin. The use of high SPF sunscreens can also provide consumers a margin of safety against erythema when they under-apply, as shown in previous work (19).

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