Sun protection products protect against UV-induced mitochondrial DNA damage in human dermal fibroblast skin cells. E-poster number P2945

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Background

Many studies have implicated the key role of mitochondria (the batteries of the cell) in the process and mechanism of ageing, particularly the role of oxidative stress and mitochondrial dysfunction in both normal skin ageing and skin photoaging[1-4]. Mitochondrial DNA (mtDNA) has been established as a reliable and sensitive biomarker of UV-induced damage in the skin. This is due to its absence of protective histones, limited repair mechanisms, and its presence as multiple copies within a cell[5]. A quantitative real-time PCR (qPCR) strand break assay of a specific "1kb UV-hotspot damage region" is used to measure mtDNA damage as qPCR amplification efficiency is decreased in the presence of high levels of UV-induced mtDNA damage.

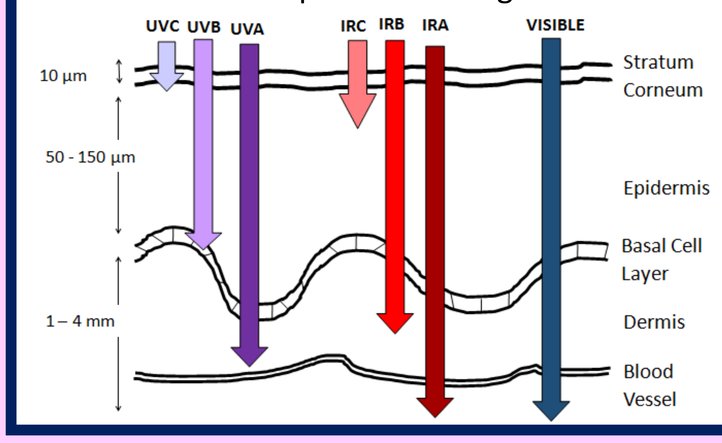

Figure 1. Penetrative capabilities of UVA and UVB on the skin in comparison to other wavelengths. UVB (280-320nm) exerts effects on the epidermal layer, whilst UVA (320-400nm) can penetrate into the dermis

Project Aim

Do currently available sun protection products provide protection by reducing the amount of UV-induced mitochondrial (mtDNA damage) in human dermal fibroblast skin cells.

Methods

Four different commercial sun protection products (products 1 to 4 in figure 2), all SPF 50) at 2mg/cm^2 were suspended on a UV transparent support between the cultured human dermal fibroblast skin cells and the UV source. The skin cells were irradiated in 35mm dishes with a physiological UV dose which is equivalent to 2 standard erythemal doses (SED) of UV light. SED is not linked to skin type, unlike minimal erythemal dose (MED), and is therefore skin type independent, the weighted measurement of sun exposure is equivalent to 100 Jm^2. The UV solar lamps used are Cleo performance 100W-R. To represent 100% protection (negative control), dishes were wrapped in aluminium foil, and to represent 100% exposed, no product was used (positive control). A sham control was a cream with no SPF.

Following irradiation DNA was extracted from the cells and analysed by qPCR. The mtDNA damage qPCR assay of a 1kb UV-hotspot region was determined in triplicate for each of the three biological repeats of the individual sun protection products. An additional 83bp qPCR mtDNA assay was performed for each condition to ensure equal loading of mtDNA in the damage qPCR assay[5].

Results: Mitochondrial DNA damage Analysis

- The higher the Ct value on the y-axis of figure 2, the greater the amount of mtDNA damage. As expected, mtDNA damage was significantly greater ($P<0.0028$) when the cells were exposed to 2 SED solar UV (100% exposed, positive control) compared to when the cells were completely foil-covered (100% protected, negative control).
- However, the key finding was that all four sun protection products showed a similar degree of UV protection of mtDNA compared to the 100% exposed cells, as seen by decreasing the level of mtDNA damage. This degree of protection (expressed as Ct values) equates to 3.2 fold less mtDNA damage, or 320% protection, compared to exposed cells and this was statistically significant for all four products ($P\leq0.02$, see table). All 4 SPF products show statistically significant protection of UV-induced mtDNA damage.

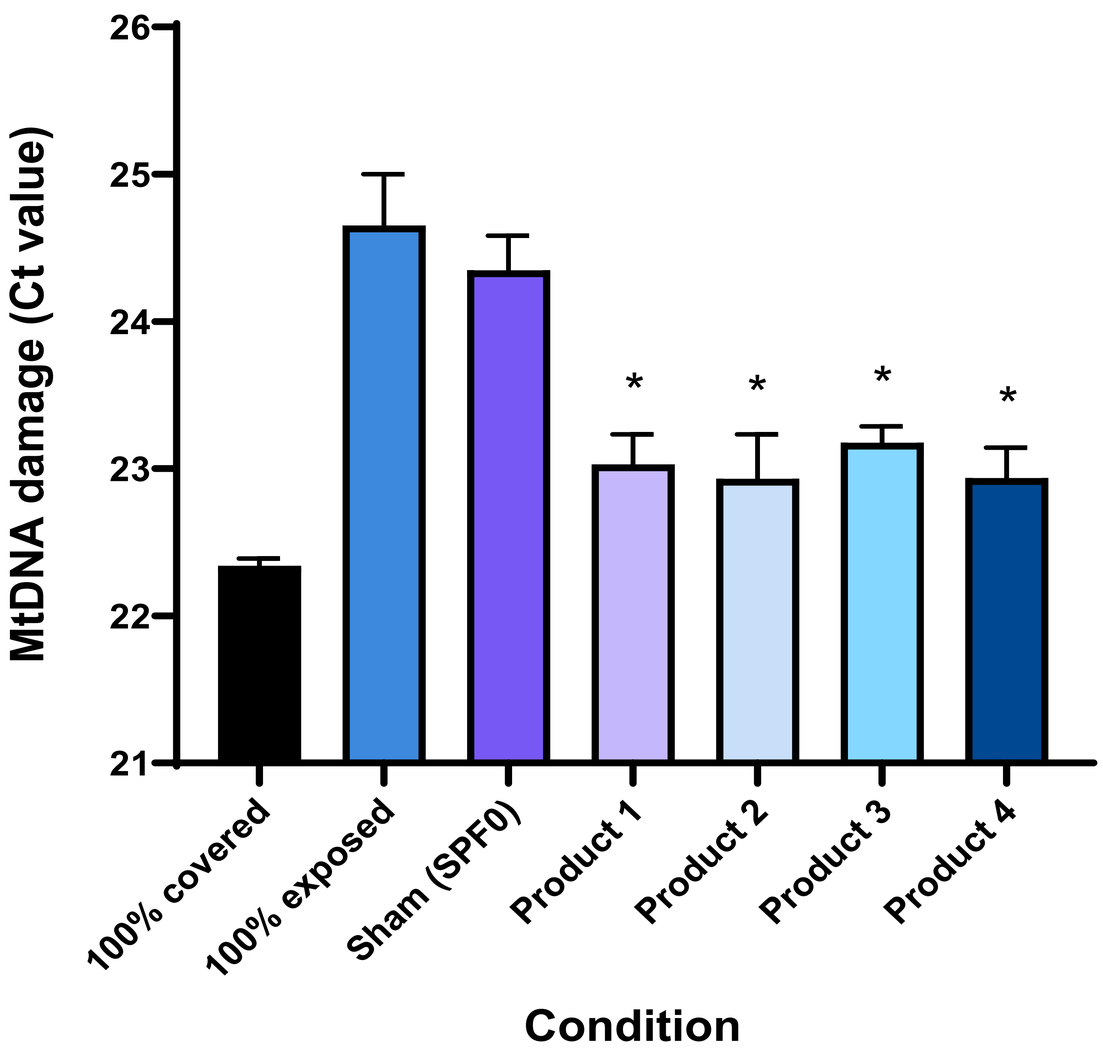

Figure 2. Mitochondrial DNA damage differences following UV irradiation in the presence of the different SPF products. Cells were irradiated in 35mm dishes using 2 SED UV light. To represent 100% covered, dishes were wrapped in aluminium foil, and to represent 100% exposed, Transpore tape on the dish with no cream was used. The sham was a control cream with no SPF. All 4 SPF 50 products were each applied to the Transpore tape at 2 mg/cm^2. Three biological repeats were performed for each condition, with the PCR run in triplicate for each sample, therefore each column represents 9 data point**s.**

Unpaired t-test to compare to 100% exposed sample:

Condition	*p*-value
100% exposed vs 100% covered	0.0028**
100% exposed vs Sham (SPF0)	0.5102
100% exposed vs Product 1	0.0161*
100% exposed vs Product 2	0.0203*
100% exposed vs Product 3	0.0157*
100% exposed vs Product 4	0.0133*

Conclusion

This set of experiments investigated four commercial sun protection products (all SPF 50) in terms of their potency of protection against UV-induced mtDNA damage with the products in between the light source and the human skin cells. The statistically significant results clearly demonstrate in cultured human skin cells, the high potency of protection of all four SPF products against UV-induced mtDNA damage which is an established biomarker of UVR damage in human skin ageing. This finding shows that the SPF products protect against damage to the DNA housed inside the batteries of the cell thereby helping to combat skin fatigue and promote increased bioenergy in human skin.

References

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