Do We Also Face Hazards Triggered By Sub-Erythemal UV Exposure? Results of a Clinical Study on Various Biological Endpoints

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Repeated sub-erythemal UV exposure on human skin may occur during short periods of UV exposure while performing non extensive outdoor activities. This may also occur during extensive UV radiation exposure while on holidays through improper use of sun protection combined with the use of low protection factors. The effects of such repeated radiation are barely investigated. There are no visible signs of skin redness in this case, however, we were asking: is there significant damage going on which would call for intervention? In particular we were interested whether the immune status of the skin is already compromised after sub-erythemal radiation and if this could be prevented through application of sunscreens.

We studied the effects of 11 consecutive daily sub-erythemal exposures of solar simulated radiation (SSR) on buttock skin of 6 healthy sun sensitive skin types I/II (18-35 years). A standard dose was given for each exposure which represented 0.52 or 0.65 minimal erythema doses (MED) depending on the MED of the volunteer. Erythema was assessed daily and biopsies were taken to assess end-points relevant in regards to the formation and propagation of skin cancer.

We also evaluated the effects of a broad-spectrum (4* UVA) daily-care low SPF (7.5) sunscreen with 6% Polysilicone-15 (PARSOL® SLX) as a UVB filter and 2% Butyl Methoxydibenzoyl-methane (PARSOL® 1789®) as a UVA filter. The study, approved by the Ethics Committee of St Thomas' Hospital London, was done according to the Declaration of Helsinki. For 11 consecutive days 6 volunteers were exposed each day to a radiation of 0.6 MED. Biopsies were taken at day 0, 5, 11 and 12. CD1a served as marker to investigate the presence of Langerhans cells (LC). In addition, we checked skin redness, quantified thymine dimers, p53 expression, the proliferation markers MIB-1, and searched for the indicators of apoptosis: BCL-2 and sun burned cells (SBC). There where two test sites on the skin: one with and one without a broad spectrum sunscreen applied.

Results

Erythema accumulated on the vehicle control site but not on the sunscreen site (Figure 1). At day 12, the vehicle site showed a dramatic increase in the erythema index and considerably more DNA damage than the sunscreen site (Figure 2). Overall, sunscreen treated sites showed much less DNA damage than the vehicle sites at all time-points ($p < 0.01 - 0.03$).

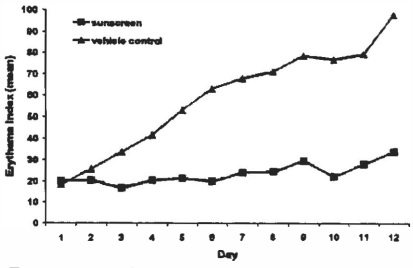

Figure 1 Erythema Index

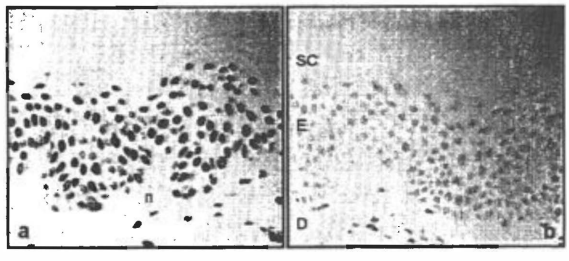

Figure 2: Immunostaining of thymine dimers.
a: placebo control, **b:** sunscreen protected; SC: stratum corneum, E: epidermis; D: dermis; n: nuclear staining

P53 expression was very low on all sunscreen treated sites. Much higher expression was seen on the vehicle sites but with high inter-person variation. Significant protection ($p < 0.01$) was seen only at day 11. There was no difference in BCL-2 expression between the 0MED control and any other site and no sunburned cells were seen on any site. Regarding the immune status we saw a time-dependent trend for depletion of LC with the vehicle that was significantly different from 0 MED and the sunscreen treated sites at days 11 and 12 (Figure 3).

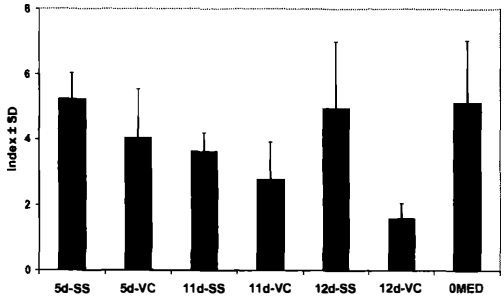

Figure 3: Sunscreen (SS) application prevents LC depletion analyzed by CD1a as marker. (VC) = vehicle control

The sunscreen protected sites were not significantly different for LC from the not radiated control at any time point. There was a high inter-personal variation within the epidermal proliferation marker (MIB1), even with the not radiated control site. Sunscreen values were lower but not significantly so. In line with MIB1 the mean number of viable cell layers was not affected by any treatment as well.

Conclusions

Despite the low radiation dosis thymine dimers were formed Jans et al. showed in 2005 that thymine dimmers are the basis for skin mutations and could lead to skin cancer. Comparisons at all 3 time points showed a very significant (p generally < 0.01) reduction of thymine dimers in sunscreen groups compared with their vehicle controls. There is considerable evidence that the thymine dimers in particular initiate erythema and our data support this.

The cellular response in addition to thymine dimer formation is regulated by p53 with two competing protection mechanisms: DNA repair or apoptosis (SBC) (Melnikova and Ananthaswamy, 2005). Unfortunately in our study we surprisingly saw no induction of apoptosis or formation of SBCs.

The depletion of antigen presenting Langerhans cells, along with the influx of Cb11b macrophages is important in the immunomodulatory effects of UVR (Meunier et al., 1995). The vehicle control data show a time-dependent depletion of LC that was totally prevented by the sunscreen.
The MIB1 data suggest that all treatments continue their epidermal proliferation though the large interpersonal variation meant no treatment was significantly different from any other or from the no radiation control. This was supported by the epidermal layer data. These data indicated that a protective mechanism such as a stop of epidermal proliferation failed to be triggered.

In conclusion, daily sub-erythemal SSR exposure in skin types I/II clearly resulted in clinical, cellular and molecular damage. Much of this damage, and in some cases all of it, can be inhibited already by a low SPF sunscreen that may be effective in the prevention of long-term photo damage, including skin cancer. It is worthwhile to note that particularly sub-erythemal radiation is able to cause skin damage; however, some protective mechanisms like proliferation stop, apoptosis and epidermal thickening are not triggered. Therefore, in conclusion, it is recommended to use UV protection also in daycare, as well as to animate the consumer to use sufficient application amounts and higher SPF's in recreational sun care.