

UV-induced Alterations in Skin and Lymphocytes during a One-week Holiday in the Canary Islands in May

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The effect of solar exposure during a one-week sunbathing vacation in May at 29°N latitude was investigated in 22 volunteers. The following end-points were measured before and after the holiday: skin reflectance at 507 nm, transmission of radiation from 289 nm to 448 nm through the epidermis, epidermal thickness, minimal erythema dose (MED), total white cell, neutrophil, lymphocyte, and lymphocyte subpopulation counts in blood, spontaneous DNA synthesis, DNA strand breaks and sister-chromatid exchange in lymphocytes, and the UVC tolerance of lymphocytes. There was a statistically significant decrease in skin reflectance ($p < 0.001$) and epidermal transmission ($p < 0.01$) after the holiday, reflecting increased pigmentation and epidermal thickening. There was a statistically significant increase in epidermal thickness ($p < 0.001$), MED ($p < 0.05$), spontaneous DNA synthesis ($p < 0.01$) and DNA strand breaks in lymphocytes ($p < 0.02$) after the holiday. The other end-points were not significantly changed. We report that changes in skin pigmentation and epidermal thickness occur after one week of UV exposure. It was also observed that a one-week UV exposure increased both spontaneous DNA repair and the amount of DNA strand breaks in the lymphocytes of the volunteers, while no changes in T-cell subsets were detected. **Key words:** Pigmentation; Minimal erythema dose; Sister-chromatid exchange; DNA strand breaks.

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Exposure to ultraviolet (UV) radiation is the principal cause of non-melanoma skin cancer, which is one of the most frequent tumors occurring in Caucasians in northern Europe and the United States (1). The increased incidence of non-melanoma skin cancers is believed to be connected with changing social habits, with increased exposure to sunlight (2). Most people in northern Europe and the United States are indoor workers. They get most of their UV exposure in the weekends and when they are on vacation (2). During the last 30 years it has become popular among people in northern Europe to spend the holiday in the Mediterranean region and in the Canary Islands (2). It has been calculated that an annual sunbathing vacation in the Mediterranean area may increase the risk of skin cancer with a factor of 5 (2). Therefore this type of recreational sun exposure seems to be an important factor in contributing to the overall risk of skin cancer.

Exposure to UV radiation induces sunburn, pigmentary and texture changes in the skin, DNA damage and immunological alterations and increases the risk of skin cancer (3–5). Some of

these effects are acute, while others are associated with chronic sun exposure. The purpose of this study was to evaluate the effects of recreational UV exposure during a one-week holiday in May in the Canary Islands at 29°N latitude on skin pigmentation, photoprotection, DNA in lymphocytes and the immune system.

MATERIAL AND METHODS

Subjects and experimental procedure

Twenty-two healthy volunteers (age 20–47 years) were enrolled in the study after giving informed consent; 10 subjects were skin types I and II, 12 subjects were skin types III and IV. All volunteers were sent on a one-week holiday at the beginning of May in the Canary Islands at 29°N latitude. The volunteers were requested to stay in the sun as long as possible. Volunteers with skin types I and II spent from 0.5–3 h in the sun every day. They generally avoided sunbathing from 11 am to 3 pm. Volunteers with skin types III and IV spent from 1–4 h in the sun every day. The weather during the experiment was only moderately good with noon temperatures around 20°C, and a majority of slightly overcast days. The estimated daily sun exposure was 0.25–4 erythemal units (6). None of the volunteers experienced an erythema during the vacation.

The parameters were measured before and after the holiday. All parameters, except UV tolerance of the lymphocytes, DNA synthesis and DNA strand breaks in the lymphocytes, were measured within 3 days after the end of the holiday. The last three parameters were measured within 2 weeks after the holiday.

Assessment of tan

Skin pigmentation of the inside of the forearm was measured by skin reflectance at 507 nm. A Xenon lamp was filtered by using a single monochromator (Jobin Yvon H20, France) and connected to a bifurcated light guide. The reflection was measured by a radiometer (EG & G 550, Salem, MA, USA) with a calibrated multiprobe detector (EG & G 550-2).

Radiation transmission through epidermis

Epidermal samples from the inside of the forearm with a diameter of 2 cm were obtained by producing a suction blister. The transmission of the epidermis was measured with a Xenon lamp, a single monochromator (Jobin Yvon H20) and a radiometer (EG & G 550) with a calibrated multiprobe detector (EG & G 550-2) at 289, 301, 310, 319 and 448 nm.

Epidermal thickness

Epidermal samples from the inside of the forearm with a diameter of 2 cm were obtained by producing a suction blister. The samples were examined histologically after hematoxylin-eosin staining. The thickness of the epidermis was determined in 20 regularly distributed sections of each sample, and all slides were read blindly.

Minimal erythema dose

Minimal erythema dose (MED) was assessed by exposure to a bank of Bellarium-S SA-1-12 tubes. The UV radiation was administered

Table I. The influence of solar exposure on the skin during a one-week holiday in May at 29°N latitude

n = number of volunteers tested

Skin parameter	<i>n</i>	Before holiday mean ± SD (range)	After holiday mean ± SD (range)	Results of statistical testing
Skin colour: reflection % at 507 nm	13	82.9±7.5 (68–95) 100%	72.5±9.8 (65–94) 87.5%	<i>p</i> < 0.001
Epidermal transmission in % at 289, 301, 310, 319 and 448 nm	10	289 nm: 8.4±2.2	4.0±0.9 47.6%	<i>p</i> < 0.002
		301 nm: 22.8±3.6	14.4±2.9 63.2%	<i>p</i> < 0.002
		310 nm: 29.4±3.6	20.9±3.4 71.1%	<i>p</i> < 0.002
		319 nm: 31.8±3.3	24.2±3.6 76.0%	<i>p</i> < 0.01
		449 nm: 66.2±4.2	61.7±4.7 93.3%	NS <i>p</i> < 0.1
100%				
Epidermal thickness (µm)	16	55.2±8.7 (34–69) 100%	80.4±16.5 (50–108) 145.7%	<i>p</i> < 0.001
Minimal erythema dose (J/cm ²)	11	29.5±12.6 (14–55) 100%	38.5±12.5 (27–59) 130.5%	<i>p</i> < 0.05

through an automatic closing shutter to the inside of the forearm. The erythema was evaluated clinically 24 h later. MED was determined as the energy needed to elicit a clear erythema in the test area.

Immunological studies

Blood immune status was assessed by measurements of total white cell, neutrophil, lymphocyte and T-lymphocyte subsets. T-lymphocyte subsets were measured with monoclonal antibodies (Becton Dickinson) against CD5 (Pan T-cells), CD4 (helper T-cell), and CD8 (suppressor T-cell) antigens.

Sister-chromatid exchange (SCE)

Culture and preparation of lymphocytes for sister-chromatid exchange (SCE) were performed as described by Wulf (7). Thirty cells were analyzed for each person before and after the holiday and all slides were read blindly.

UV tolerance of the lymphocytes

The isolation, irradiation and incubation of the lymphocytes were performed as described by Munch-Petersen et al. (8). The UV tolerance of the lymphocytes was estimated by the UVC dose (J/m²)

necessary for a 50% reduction in phytohaemagglutinin (PHA)-stimulated lymphocyte proliferation assessed after 7 days of growth.

DNA synthesis in lymphocytes

The spontaneous DNA synthesis, i.e. non-replicative DNA synthesis, was determined as the amount of [Me-³H]-thymidine (10 Ci/ml, 25 µCi/mmol) incorporated in lymphocytes in vitro during 1 h of incubation in RPMI supplemented with 20% fetal calf serum at 37°C (8). The results were expressed as counts/min/10⁶ cells/h.

DNA strand breaks in lymphocytes

An estimate of the amount of DNA strand breaks was obtained by a fluorimetric alkaline unwinding test (9) and expressed as the relative amount of double stranded DNA present after partial unwinding of DNA for 1 h at 18°C at pH 12.8.

Statistics

The Wilcoxon matched-pairs signed rank test was used for comparison of the end-points before and after the holiday. The Mann-Whitney U-test was used to compare the changes in the fair-skinned volunteers with the changes in the rest of the volunteers. The correlation between

Table II. The influence of solar exposure on the lymphocytes during a one-week holiday in May at 29°N latitude

n = number of volunteers tested

Lymphocyte parameter	<i>n</i>	Before holiday mean ± SD (range)	After holiday mean ± SD (range)	Results of statistical testing
Helper T-cells (10 ⁶ /ml)	22	1.22±0.42 (0.49–2.41) 100%	1.18±0.44 (0.51–1.91) 96.7%	NS
Helper/suppressor T-cell ratio	22	2.05±0.69 (0.89–3.26) 100%	2.01±0.70 (1.00–4.00) 98.0%	NS
Sister-chromatid exchange (SCE)	17	6.59±1.04 (5.07–8.00) 100%	7.17±1.08 (5.53–9.00) 108.8%	NS 0.05 < <i>p</i> < 0.1
UV tolerance (J/cm ²)	11	3.4±0.8 (2–4.3) 100%	3.0±0.8 (2–4) 88.2%	NS 0.1 < <i>p</i> < 0.2
Spontaneous DNA synthesis ^a (cpm/10 ⁶ cells/h)	21	782±422 (276–1754) 100%	1080±461 (462–2102) 138.1%	<i>p</i> < 0.01
DNA strand breaks ^b	22	86.4±6.2 (75–98.5) 100%	82.3±4.2 (70.8–89) 95.3	<i>p</i> < 0.02

^ai.e. non-replicative DNA synthesis

^bdouble-stranded DNA in % of total DNA

skin reflectance and MED was investigated by Spearman's test for rank correlation. In all test the results were considered significant when $p < 0.05$.

RESULTS

Tables I and II show the results of the different methods used to assess UV-induced alterations during a one-week holiday. Total white cell, neutrophil and lymphocyte counts are not shown in the tables. We did not observe any significant changes in these three parameters during the holiday, $p < 0.02$.

Table I shows that the skin colour of our volunteers increased significantly during the holiday. The increase in skin pigmentation was not significantly larger among our volunteers with skin types III and IV (skin reflectance 78.9% to 67.4%, mean 11.5, range 3–17, $n = 7$) than among our volunteers with skin types I and II (skin reflectance 88.0% to 79.7%, mean 8.3, range 1–18, $n = 6$). We observed a significant increase in epidermal thickness during a one-week sun exposure, $p < 0.001$. The increase in epidermal thickness among our volunteers with skin types I and II (mean 8.9 μm , range 2.7–14.9, $n = 10$) was similar to the increase among our volunteers with skin types III and IV (mean 7.5 μm , range –6.0–17.5, $n = 6$), $p < 0.1$. Both the increased pigmentation and thickness of epidermis resulted in a decreased epidermal transmission. The largest decrease in epidermal transmission was observed at 289 nm, while the transmission at 448 nm was not significantly lower after the vacation compared with before.

Table II shows that the number of T-cells and the helper/suppressor T-cell ratio did not change during the vacation. There was an insignificant increase in SCE. The increase in SCE was not significantly larger among the fair-skinned volunteers (mean 0.50, range –1.33–2.87, $n = 9$) compared with the volunteers with skin types III–IV (mean 0.70, range –1.05–2.36, $n = 8$), $p < 0.1$. Similarly, the increase in spontaneous DNA synthesis was not significantly larger among our volunteers with skin types I and II (mean 327, range –46–754, $n = 9$) than among our volunteers with skin types III and IV (mean 271, range –661–1245, $n = 12$), $p < 0.1$. The decrease in the amount of double-stranded DNA was not larger among our volunteers with skin types I and II (mean 4.0, range –10.8–+3.8, $n = 10$) than among our volunteers with skin types III and IV (mean 3.6, range –14.2–+9.6, $n = 12$).

There was a negative relationship and a significant correlation between skin reflectance % at 507 nm and the volunteers' minimal erythema dose, $p < 0.002$.

DISCUSSION

Vacations under the intense sun of Southern Europe and Africa have become increasingly popular among the rather sun-sensitive individuals from Northern Europe. Many people believe that sunlight is beneficial to their well-being, and despite warnings, most individuals enjoy lying in the sun. However, most people with sensitive skin are aware of their intolerance to UV exposure and therefore tend to reduce solar exposure and protect themselves better (10). Our study included subjects with sensitive skin and subjects with more sun-resist-

ant skin. We did not find that the UV-induced alterations were significantly higher in our group of fair-skinned subjects compared to those with a more sun-resistant skin. All volunteers were requested to stay in the sun as long as possible, but they were allowed to adjust their solar exposure during the holiday according to their own wishes. This enabled the more fair-skinned subjects to reduce their solar exposure. This study indicates that people with sensitive skin, probably due to their acquired sunbathing habits, may not run a greater risk of suffering UV-induced alterations during a one-week holiday than people with skin types III and IV. However, it is important to remember that because of the weather the solar irradiance was not very high, and that none of the fair-skinned individuals suffered a burn.

In the last decade there have been many reports indicating interaction between UV surface irradiation and the lymphatic system (3–5, 12). Natural sunlight and artificial UV exposure alter the proportion of circulating helper T-lymphocytes and increase suppressor cell activity (3–5, 11). In vivo, circulating lymphocytes are affected by UV irradiation directly and indirectly. White blood cells are exposed to UV radiation when they pass through the superficial blood vessels of the dermis (13). The indirect effect is mediated through the Langerhans' cells and non-Langerhans' antigen-presenting cells (14). In this study we report on the overall effect on the number of circulating helper-T-lymphocytes and the helper/suppressor ratio. Additionally, we evaluated the direct effects of solar exposure in vivo on lymphocytes using four different end-points; SCE, spontaneous DNA synthesis, i.e. non-replicative DNA synthesis, DNA strand breaks and UVC tolerance.

Two weeks of moderate solar exposure (1 h/day) result in an increase in the number and activity of T-suppressor cells and a decrease in T-helper cells (4). A course of exposure to UVA sunbeds also decreases the number of circulating helper T-cells and the helper/suppressor ratio (3, 5). We did not observe a significant decrease in either helper T-cells or in the suppressor/helper ratio after one week of moderate UV-exposure in Southern Europe on a sunbathing holiday. This could indicate that one week is not enough to trigger UV-induced alterations, at least when no burns are suffered (15).

The SCE assay is a method to detect genetic damage (7) and is commonly used to evaluate the genotoxic potential of exogenous factors (16). In an early report it was noticed that there existed a linear relationship between UVA exposure dose and the occurrence of SCE in Chinese hamster ovary cells in vitro (17). In this study we were not able to find a significant increase in SCE in the lymphocytes of our volunteers. However, the increase was almost significant, indicating that SCE can probably be used to evaluate the genotoxic potential of higher UV exposures in humans.

Circulating lymphocytes from normal individuals are G_0 cells, and the very limited ability to incorporate radioactive thymidine, i.e. spontaneous DNA synthesis, is believed to be due to a few S-phase cells in the population. The present results show a significant increase in the spontaneous DNA synthesis after one week of moderate solar exposure ($p < 0.01$). Also, in previous investigations, an increased level of spontaneous DNA synthesis has been observed during sum-

mer periods (8). Increased DNA synthesis in G₀ cells from otherwise healthy individuals may reflect an increased repair of DNA damage resulting from sunbathing.

The relative amount of double-stranded DNA in the lymphocytes was decreased significantly ($p < 0.02$), indicating an increase in the amount of DNA strand breaks in the volunteers after one week of increased solar exposure. Parallel to the increase in spontaneous DNA synthesis, the elevated number of DNA breaks may be the result of ongoing DNA excision repair at the damaged sites in the DNA strands. This implicates, however, that the DNA repair process is surprisingly slow *in vivo*, since it is still taking place several days after solar exposure has stopped.

The UV tolerance reflects the ability of the circulating lymphocytes to survive UVC exposure *in vitro* (8). The UV tolerance of lymphocytes is directly correlated to the T-lymphocyte helper/suppressor ratio and indirectly correlated to number of NK cells (18). Earlier reports have shown that the UV tolerance of lymphocytes from skin cancer patients with a history of extensive solar exposure is significantly decreased compared with controls (18, 19). Additionally, it has been shown that the UV tolerance is 1.5-fold higher in the winter than in the summer (8). In this study we evaluated the acute effect of a moderate solar exposure in healthy subjects. We were unable to see a significant decrease in UVC tolerance of the lymphocytes. The moderate UV exposure is the most evident explanation for this. However, it is important to notice that only 1 subject out of 11 had an increased UV tolerance, while 6 had a decreased UV tolerance.

In 4 of the volunteers, 2 fair-skinned and 2 with skin type III, the UV tolerance of the lymphocytes, DNA synthesis in lymphocytes and DNA strand breaks in lymphocytes were not measured before 13 days after the holiday. In a previous study it was observed that the DNA synthesis decreased slowly after a vacation in the Mediterranean area (8). The pre-vacation level was first obtained after 3 months (8). Also the UV tolerance of the lymphocytes changes slowly (8). Therefore it is unlikely that these parameters changed significantly during the 13 days.

In this study the UV-induced alterations were evaluated in 22 volunteers during a one-week sunbathing vacation in Southern Europe. Firstly, we observed that the UV-induced alterations were not significantly higher in our group of fair-skinned subjects compared with the more sun-resistant. This probably reflects the different sunbathing habits of the two groups. Secondly, the end points reflecting direct damage of UV radiation on lymphocytes were more sensitive than changes in T-cell subsets. This study indicates that spontaneous DNA synthesis and DNA strand breaks might be sensitive indicators of minor solar-induced damage. However, it cannot be excluded that changes in alcohol consumption, smoking and diet during the vacation might induce some of the observed alterations in the lymphocytes (7).

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