The Effect of Repeated Exposures to Low-Dose UV Radiation on the Apoptosis of Peripheral Blood Mononuclear Cells

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Objective: To investigate whether repeated exposures to low-dose UV or solar-simulated radiation induce apoptosis of peripheral blood mononuclear cells.

Design: Cohort study in a healthy population.

Setting: Departments of Dermatology and Hematology, Medical University of Lodz, Lodz, Poland.

Participants: Ninety-eight healthy volunteers were divided into the following 4 groups: group A, whole-body irradiated with a 0.7–minimal erythema dose (MED) of UV-B daily on 10 consecutive days followed by a single dose of 3 MEDs on a small body area 24 hours later; group B, whole body irradiated with 120 J/m² solar-simulated radiation (10 consecutive days, then single-dose UV-B exposure of 3 MEDs on a small body area after 24 hours); group C, irradiated with a single UV-B dose of 3 MEDs on a small body area; and group D, irradiated with a single UV-B dose of 4 MEDs on a small body area.

Main Outcome Measure: Apoptosis of peripheral blood mononuclear cells as well as expression of several apoptosis-regulating proteins in response to irradiation.

Results: Ten daily whole-body suberythemal exposures to UV-B or solar-simulated radiation enhanced the apoptosis of peripheral blood mononuclear cells, while the single erythemal doses on the small body area, either on their own or following the repeated exposures, did not increase the level significantly. Increase in Bax and p73 protein expression and down-regulation of Mcl-1 and Bcl-2 correlated with enhanced apoptosis.

Conclusion: Repeated suberythemal UV exposures enhance the apoptosis of peripheral blood mononuclear cells in healthy subjects.

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Abnormal activation of apoptosis and disturbances in apoptotic cell clearance may result in decreasing immunological tolerance and, as a consequence, the production of autoantibodies and the development of autoimmune diseases. However, pathological inhibition of cell apoptosis is believed to play a crucial role in carcinogenesis.

Exposure to solar UV radiation (UVR) is the most important environmental factor in skin carcinogenesis. It is recognized to suppress cell-mediated immune responses in both animal models and human beings by a complex process initiated by chromophores located in the skin and ending with the generation of T regulatory cells. For example, we have shown previously that the contact hypersensitivity response is suppressed in subjects exposed to repeated suberythematous doses of solar-simulated radiation (SSR) or UV-B. In addition, short-term UV-B irradiation can induce the apoptosis of keratinocytes. This results in the formation of scattered dyskeratotic keratinocytes with condensed or absent nuclei and eosinophilic cytoplasm, termed sunburn cells. They can be detected in vivo 24 hours after the exposure. Several pathways can lead to the formation of sunburn cells, such as p53 protein up-regulation or signaling through the Fas/Apo1 death receptor.

Our previous studies demonstrated that repeated exposures to both low-dose UV-B and SSR are able to influence the number of circulating blood dendritic cells and alter the serum concentrations of interleukin 8 and tumor necrosis factor in healthy human subjects. These observations raised the question of whether similar exposures could induce apoptosis in circulating peripheral blood mononuclear cells (PBMCs). The present study was designed to test such a possibility using the TUNEL (TdT-mediated dUTP-biotin nick-end labeling) method and the PARP cleavage assay.

**METHODS**

**SUBJECTS**

The study group consisted of 98 healthy volunteers aged 19 to 51 (median, 27.5) years, with either skin phototype II or III, as assessed by the Fitzpatrick classification. They were without any skin or other diseases and were not receiving any medication. Before the study, blood tests (blood cell counts) and urinalysis were performed. People exposed to excessive sunlight or sunlamps within 2 months before the study began were excluded. To decrease the influence of natural solar radiation, all the procedures were performed in winter months. Each volunteer gave written informed consent before entry into the study, and the experimental plan was approved by the local ethics committee. The study was performed in accordance with the Helsinki declaration. The subjects received a thorough physical examination and blood cell count. The clinical characteristics of the volunteers are given in Table 1.

**PHOTOTESTING AND UV IRRADIATION**

Phototesting (UV-B radiation) of each volunteer to determine their personal minimum erythema dose (MED) was undertaken approximately 1 week before the experiment began using a Waldmann Medizintechnik UV 109 device (Waldmann Medizintechnik, Villingen-Schwenningen, Germany) containing B12 lamps (Philips, Eindhoven, the Netherlands) emitting 58.6% UV-B and 41.4% UV-A, with an incremental dose series on 6 squares (1 × 1 cm) on the inner surface of the forearm. The initial lowest dose varied between 0.05 and 0.09 J/cm², depending on skin phototype, and each increment was approximately 20%. The MED was defined as a just-perceptible erythema filling the irradiated site as assessed visually 24 hours later.

The volunteers were divided into 4 groups, as given in Table 1: group A contained 30 individuals who were whole-body irradiated (excluding the genital area) with 0.7-MED UV-B for 10 consecutive days, followed by a single dose of 3-MED UV-B on a small body area (left buttock, 10 × 10 cm) 24 hours later; group B contained 29 subjects who were irradiated with a 1.2-standard erythema dose (where 1 standard erythema dose is equivalent to an erythemal radial exposure of 100 J/m²) on each of 10 consecutive days, followed 24 hours later by a single dose of 3-MED UV-B on a small body area (left buttock, 10 × 10 cm); group C contained 29 individuals who were irradiated with a single dose of 3-MED UV-B on a small body area (left buttock, 10 × 10 cm); and group D contained 10 individuals who were irradiated with a single dose of 4-MED UV-B on a small body area (left buttock, 10 × 10 cm). The whole-body UV-B was generated by 100-W B12 lamps (Philips), giving an even field of irradiance (285-340 nm) of approximately 3.85 mW/cm² on the skin surface at 20 cm from the source. The SSR was generated by 100-W Cleo Natural lamps (Philips) in 2 half-cabinets, giving an even field of irradiance (4% UV-B and 96% UV-A) of approximately 4.95 mW/cm² on the skin surface 20 cm from the source. The spectrum emitted by the Cleo Natural lamp is shown in Figure 1. Measurement of the intensity of the lamps was performed using a Solar Light 3-D UV meter (Solar Light Co, Philadelphia, Pennsylvania). The Waldmann phototesting device was used to irradiate the 10 × 10-cm area of the buttock with 3- or 4-MED UV-B.

**Table 1. Clinical Characteristics of the Volunteers in Groups A Through D**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Volunteers</th>
<th>Mean Age, y</th>
<th>Sex, F:M, No.</th>
<th>Phototype II/III, No.</th>
<th>Median MED UV-B, J/cm² (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10 × UV-B, 3-MED UV-B</td>
<td>30</td>
<td>26.9</td>
<td>17:13</td>
<td>0.13 (0.09-0.13)</td>
</tr>
<tr>
<td>B</td>
<td>10 × SSR, 3-MED UV-B</td>
<td>29</td>
<td>27.9</td>
<td>14:15</td>
<td>0.14 (0.07-0.15)</td>
</tr>
<tr>
<td>C</td>
<td>3-MED UV-B</td>
<td>29</td>
<td>28.2</td>
<td>16:13</td>
<td>0.15 (0.09-0.17)</td>
</tr>
<tr>
<td>D</td>
<td>4-MED UV-B</td>
<td>10</td>
<td>26.9</td>
<td>5:5</td>
<td>0.16 (0.07-0.16)</td>
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</table>

Abbreviations: MED, minimal erythema dose; SSR, solar-simulated radiation.
BLOOD SAMPLES AND ISOLATION OF PBMCs

Heparinized blood samples were taken from the volunteers in all 4 groups before irradiation. In groups A and B, they were also collected 24 hours after the final daily exposure and again 24 hours after the single UV-B exposure, and in groups C and D, they were also collected 24 hours after the single UV-B exposure. The PBMCs were isolated by centrifugation in Histopaque-1077 (Sigma Diagnostic, St Louis, Missouri) density gradients. The interphase region containing PBMCs was collected, the cells were washed twice and then fixed in 1% methanol-free formaldehyde (Polysciences Europe, Eppelheim, Germany) for 15 minutes at 0°C, before being permeabilized in 70% ethanol (30 minutes at 0°C).

ASSESSMENT OF APOPTOSIS

For practical reasons, apoptosis was assessed at a single time, 24 hours after the final UV exposure. This point was thought most appropriate as demonstrated in studies of apoptosis in PBMCs irradiated in vitro with a range of UV-A and UV-B wave bands.22

TUNEL Assay

A modification of the TUNEL assay was used to assess DNA fragmentation (APO-BRDU kit; Phoenix Flow Systems, San Diego, California).23 In this method, the 3′-OH termini of the DNA strand breaks serve as primers and become labeled with bromodeoxyuridine (BrdU) when incubated with BrdU triphosphate in a reaction catalyzed by exogenous terminal deoxynucleotidyl transferase. The incorporated BrdU is immunocytochemically detected by BrdU antibody conjugated to fluorescein isothiocyanate (FITC). After the fixed and permeabilized cells were incubated with the APO-BRDU reagents (60 minutes at room temperature and in the dark), cellular DNA was counterstained for 30 minutes (at room temperature and in the dark) with a mixture of 5-mg/mL propidium iodide and 100-mg/mL RNase A (DNase-free) (Sigma Aldrich, Brodby, Denmark). Then, cell green (FITC) vs red (propidium iodide) fluorescence isothiocyanate (FITC). After the fixed and permeabilized cells were incubated with the APO-BRDU reagents (60 minutes at room temperature and in the dark), cellular DNA was counterstained for 30 minutes (at room temperature and in the dark) with a mixture of 5-mg/mL propidium iodide and 100-mg/mL RNase A (DNase-free) (Sigma Aldrich, Brodby, Denmark). Then, cell green (FITC) vs red (propidium iodide) fluorescence was measured by dual-color flow cytometry. The percentage of TUNEL-positive cells was expressed as an apoptotic index.

PARP Cleavage

Detection of the PARP cleavage product, p89, is a hallmark of effector caspase activation during apoptosis.24 The fixed and permeabilized PBMCs were incubated with the PARP primary rabbit antibody (Santa Cruz Biotechnology Inc, Santa Cruz, California), used at a dilution of 1:1000 (overnight at 4°C) before being incubated for 2 hours at room temperature with secondary anti-rabbit IgG alkaline phosphatase–conjugated antibody (NeoMarkers Inc, Fremont, California). Cell fluorescence was measured subsequently by flow cytometry.

EXPRESSION OF APOPTOSIS-REGULATING PROTEINS

The PBMCs were fixed as described in the “Blood Samples and Isolation of PBMCs” subsection before being permeabilized with 0.1% polysorbate 20 (Tween 20) in phosphate-buffered saline (Amersham Biosciences, Freiburg, Germany) immediately prior to the protein expression measurements. The Bcl-2 family members (Bax, Bak, Bcl-2, and Mcl-1) and the p53 family members (p53 and p73) were assessed. The primary antibodies used, their dilutions, and time of incubation were the following: mouse anti-p53 FITC-conjugated monoclonal antibody (clone DO-7; DAKO, Glostrup, Denmark), 1:30 dilution and 30-minute incubation time; mouse anti-Bcl-2, FITC-conjugated monoclonal antibody (DAKO), 1:15 dilution and 30-minute incubation time; mouse anti-p73 FITC-conjugated monoclonal antibody (NeoMarkers Inc, Fremont, California), 1:10 dilution and 30-minute incubation time; rabbit anti-Bax antibody (DAKO), 1:400 dilution and 60-minute incubation time; and rabbit anti–Mcl-1 antibody (Calbiochem-Novabiochem Corporation, San Diego, California), 1:500 dilution and 60-minute incubation time. Secondary swine anti-rabbit or goat anti-mouse antibodies were used at dilutions ranging from 1:20 to 1:50, as appropriate, with a 30-minute incubation time. All incubations were performed at room temperature, and, in the case of the fluorochrome-labeled antibodies, in the dark. Protein expression was measured by flow cytometry and was assessed as mean fluorescence intensity.

FLOW CYTOMETRY

All fluorescence measurements were performed by flow cytometry (FACScan; Becton-Dickinson Biosciences, San Jose, California), using standard emission filters and the CellQuest software (Becton Dickinson Biosciences) was used to process the data. Ten thousand cells per sample were processed routinely in all experiments.

STATISTICAL ANALYSIS

For the statistical analysis of data obtained, the range of the measured variable and the median (SD) values were calculated, using statistical software (STATISTICA, version 5.0; StatSoft Inc, Tulsa, Oklahoma). The data are presented as the medians (SDs) of the multiple experiments. The nonparametric Wilcoxon pair test was used to compare the results before and after irradiation. The correlation between features was evaluated using the Spearman R test. Comparisons and correlations were considered significant when P < .05.

RESULTS

Repeated low-dose exposures to UV-B or SSR did not result in reddening of the skin or edema, but a slight increase in pigmentation was observed. The single high-dose UV-B exposure induced well-defined erythema, in some cases with blistering if given on its own or following the repeated exposures.

The PBMCs were examined for apoptosis 24 hours after the final irradiation by the TUNEL and PARP cleavage methods. Because the apoptotic index assessed by the
group A (following 10 repeated UV-B exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in group B (following 10 repeated SSR exposures) compared with a significantly higher apoptotic index of PBMCs in 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group B (follow
The repeated whole-body suberythemal UV-B or SSR exposures enhanced apoptosis in the PBMCs, although there is evidence that this type of irradiation can initiate significant changes in immune responses both locally and systemically. In conclusion, our results have shown that repeated low-dose UV exposures of healthy subjects can lead to an increase in apoptosis of circulating PBMCs. Whether this change contributes significantly to the immunosuppression that follows UVR is not known at this stage, and further studies are required. In addition, this study adds to others on the potentially harmful effects of even small doses of UVR on the human immune system.

REFERENCES