Sunlight and vitamin D affect DNA damage, cell division and cell death in human lymphocytes: a cross-sectional study in South Australia

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The ultraviolet (UV)-B spectrum in solar UV radiation is essential for stimulating the epidermal production of vitamin D but also damages DNA and causes cancer in exposed cells. We examined the role of solar UV in inducing DNA damage in blood lymphocytes and the possible modulation of this damage by serum 25-hydroxy vitamin D (25(OH)D) in 207 male and female participants from South Australia. Personal solar UV exposure was estimated from hours of outdoor exposure recalled at the time of blood collection for analysis of DNA damage in lymphocytes, using the cytokinesis-block micronucleus cytome (CBMN-cyt) assay and of serum 25(OH)D. We examined the association between solar UV exposure, serum 25(OH)D and DNA damage using multiple linear regression, with age, sex, body mass index and alcohol consumption as covariates. The frequency of cells with micronuclei (a biomarker of chromosome breakage or loss) increased with increasing sun exposure [% increase = 5.24; 95% confidence interval (CI): 0.35 to 10.37 P-value = 0.04] but cells with nucleoplasmic bridges (a biomarker of misrepair of DNA strand breaks or telomere end fusions) decreased [% increase = −8.38; 95% CI: −14.32 to −2.03 P-value = 0.01]. There was also a fall in the nuclear division index (NDI) [% increase = −1.01; 95% CI: −2.00 to 0.00 P-value = 0.05], suggesting diminished mitogenic response and, possibly, immune suppression. There was no overall relationship between 25(OH)D and DNA damage. There were, however, weak modulating effects of 25(OH)D on the associations of solar UV exposure with micronucleus formation and with NDI (P-interaction = 0.03 and 0.05, respectively), where the increase in micronuclei and fall in NDI with increasing solar UV were greater at serum 25(OH)D ≤50 nmol/L. Thus, the influence of solar UV exposure in causing DNA damage or immune suppression in internal tissues may be stronger when vitamin D levels are low.

Introduction

Sunlight is both beneficial and detrimental to health. The best understood benefits arise from the ability of solar ultraviolet (UV)-B radiation to initiate epidermal vitamin D production (1). In addition to its effects on bone and muscle development and maintenance, vitamin D is anti-proliferative, pro-differentiating and pro-apoptotic (2). These properties are pivotal in maintaining cell integrity as they promote differentiation of healthy cells, while inhibiting the proliferation and propagation of damaged or mutated cells (3).

Over exposure to solar UV radiation has detrimental effects. Both of its components, UVA and UVB, can induce immune suppression and cause DNA damage, consequently compromising DNA stability (4). These wavelength bands can work synergistically in inducing these effects even at very low exposure levels (5). While most UV-induced DNA damage occurs in the epidermis of the skin, UVA penetration into the dermis causes oxidative DNA damage, which can lead to gene mutation (6). However, UV-induced DNA damage may not be limited to skin or other superficial cells. Seasonal variation in genetic changes in circulating lymphocytes suggests that sunlight can cause DNA damage in at least blood cells in cutaneous capillaries as they pass through the outer layer of the skin (7–9).

No study has yet addressed the possible relationship between sun exposure, DNA damage in lymphocytes and vitamin D. The aim of the current study was to investigate the association between personal sun exposure and DNA damage in blood lymphocytes and to see whether corresponding levels of serum 25-hydroxy vitamin D (25(OH)D), the most commonly used measure of vitamin D sufficiency, modified any observed relationship. The cytokinesis-block micronucleus cytome (CBMN-cyt) assay in lymphocytes was used as a multiple set of biomarkers of DNA damage and cytotoxicity because of its sensitivity to a wide range of genotoxic agents including UV radiation (10,11). The micronucleus index in this assay is also predictive of cancer risk (12,13).

Materials and methods

Study participants

Participants were 207 healthy male and female volunteers aged 25–60 years and residing in Adelaide, South Australia. They included people identified through the Australian Electoral Roll, who were invited by a letter to participate, and people who responded to an advertisement inviting participation in a randomised controlled trial to test the effects of a nutritional polypill on lymphocyte DNA damage. People were ineligible to participate if they were: current cigarette smokers or had smoked within the past 6 months; women who were pregnant or planning a pregnancy or taking daily dietary supplements exceeding 50% of the recommended daily allowance for any nutrient.

Participants gave signed consent to complete a questionnaire, to give a blood sample and to meet study requirements. At their first clinic visit, on which this analysis is based, participants completed a questionnaire, which included...
questions on demographic characteristics, diet, alcohol intake, hours spent outdoors between 9 am and 5 pm in each of the preceding 16 weeks, sun protection behaviour and sun sensitivity. They also gave a blood sample and their height and weight were measured. These visits were completed from late January to mid-May 2008.

The University of Sydney Human Research Ethics Committee and CSIRO Health Sciences and Nutrition Human Experimentation Ethics Committee approved the study.

Measurements in blood

The fresh blood samples, collected into heparin tubes, were used for the measurement of plasma vitamin D and to perform the CBMN-cyt assay in lymphocytes. Plasma was separated by centrifugation and stored frozen at −80°C until all samples were collected and then they were delivered frozen on dry ice to the analytical laboratory for vitamin D analysis. Vitamin D assays were done using an enzyme immunooassay (BEST 2000 Assay). This assay had inter-assay variation of 6.6% and intra-assay variation of 5.9%, which was determined by the laboratory that carried out the vitamin D assay.

The CBMN-cyt assay was performed using the isolated lymphocyte culture protocol described in detail previously (14). Lymphocytes were isolated by Ficoll separation and washed once in Hank’s balanced salt solution. The cultures were

Data analysis

Participants’ solar UV exposure in each of the 16 weeks before blood collection was calculated from their recalled hours of exposure and the average ambient UV irradiance between 9 am and 5 pm at their location in that week. Cumulative ambient UV irradiances expressed as standard erythemal doses (SED; one SED is 100 J/m² of erythemally effective UV based on the CIE erythemal action spectra) are measured daily in Australia’s major cities. The Australian Radiation Protection and Nuclear Safety Agency (ARPANS) provided average daily measurements for the period 8 am to 5 pm for each day from January 2008 to May 2008. Participants’ exposure for each week was estimated assuming that each reported hour outdoors entailed exposure to one-ninth of the average cumulative daily ambient UV irradiance in their nearest major city during that week.

Average estimated weekly solar UV exposure over the 6 weeks before blood collection was used for the analysis. This exposure period was chosen because we have shown, in this cohort, that solar UV exposure in each of these 6 weeks contributed to the variance in serum 25(OH)D levels (V. Nair-Shalliker, M. Clements, M. Fenech and B. K. Armstrong, unpublished results).

Data on skin colour and ability to tan and, separately, hat use, sunscreen use and clothing coverage were combined using factor analysis (PROC FACTOR in SAS) to form sun sensitivity and sun protection scores. Each factor analysis assumed a single latent factor and the factor scores had a mean of zero and a variance of unity. The scores were divided into quartiles that were given values of 0.25, 0.50, 0.75 and 1.00. For sun sensitivity, the lowest score represented those with high sun sensitivity (defined as ‘fair skin, not easily tanned’) and the highest score those with low sun sensitivity (defined as ‘dark skin and easily tanned’). For sun protection, the lowest score (0.25) represented those with greatest sun protection (always use a hat, apply sunscreen to all or most exposed skin and wear clothing covering all or most skin when outdoors on a sunny day and not under any shade) and the highest score (1.00) those with least sun protection (never use a hat or apply sunscreen to all or most exposed skin or wear clothing covering all or most skin when outdoors on a sunny day and not under any shade). Weekly solar UV exposure was multiplied by the sun protection score to weight it for sun protection behaviour.

Univariate and multiple variable linear regression analyses were done using SAS software (version 9.1) with the log of DNA damage and cytotoxicity biomarkers and NDI as dependent variables and the log of weekly solar UV exposure and serum 25(OH)D as independent variables. Age and sex were included as covariates in all adjusted analyses. Body mass index (BMI), fitted by (2

Results

Associations of sun exposure and vitamin D with DNA damage and other biomarkers

The median values and ranges for each of age, sun exposure, serum vitamin D, BMI and the components of the CBMN-cyt assay are shown in supplementary Table 1, available at Mutagenesis Online.

The results of fitting multiple linear regression models to the associations of log (solar UV exposure) with log (DNA damage or cytotoxicity biomarkers), with adjustment for possible confounding by age, sex, BMI and alcohol intake, are shown in Table 1. A doubling in solar UV exposure was associated with an estimated 5.20% increase in BN-MN [95% confidence interval (CI): 0.35 to 10.37; P-value = 0.04]. It was, however, associated with a reduction in the frequency of BN-NPB and necrotic cells, by 8.38 and 4.43%, respectively (95% CI: −14.32 to −2.03; P-value = 0.01 and 95% CI: −8.63 to −0.03; P-value = 0.05, respectively). A doubling in solar UV
exposure was associated with an estimated reduction of 1.01% in the NDI (95% CI: -2.00 to 0.00 P-value = 0.05). There was no association between UV exposure and BN-NBUD or apoptosis. Sun sensitivity showed no association with any of the biomarkers. Weighting estimated solar UV exposure by the sun protection score showed no important change in its associations with the biomarkers (data not shown).

Although 25(OH)D is subject to seasonal variation (15,16), there was no important variation in mean 25(OH)D levels in the months (late-January to mid-May) when blood was drawn in our cohort (P-value = 0.57) (V. Nair-Shalliker, M. Clements, M. Fenech and B. K. Armstrong, unpublished data). There was little evidence of an association between log serum 25(OH)D concentration and log-transformed frequency of any CBMN-cyt assay biomarker across the full range of 25(OH)D concentrations (P-values > 0.3 for all DNA damage biomarkers in Table I).

**Modification of sun exposure associations with DNA damage and other biomarkers by serum 25(OH)D concentration**

The possibility that serum 25(OH)D modifies the association between solar UV exposure and DNA damage biomarkers was also investigated (Table II). There was a 7.24% (95% CI 2.01 to 12.75) increase in BN-MN with a doubling in sun exposure when serum 25(OH)D levels were <50 nmol/l (insufficient), but a smaller increase, 4.75% (95% CI 0.06 to 9.67) when serum 25(OH)D levels were 50 nmol/l or higher (sufficient) (Pinteraction = 0.03; Table II). There was also evidence, although weaker, that the fall in NDI with increasing sun exposure was greater when serum 25(OH)D was insufficient than when it was sufficient (Pinteraction = 0.05; Table II).

### Table I. Linear regression analyses of log-transformed CBMN-cyt assay biomarker frequencies on log solar UV exposure and on log 25(OH)D levels in serum

<table>
<thead>
<tr>
<th>CBMN-cyt assay biomarker</th>
<th>Solar UV exposure (mJ/cm²) (n = 207)</th>
<th>Total 25(OH)D (nmol/l) (n = 207)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted for age and sex</td>
<td>Adjusted for age, sex, BMI and alcohol intake</td>
</tr>
<tr>
<td></td>
<td>% Change</td>
<td>95% CI</td>
</tr>
<tr>
<td>BN-MN</td>
<td>5.43</td>
<td>0.51 to 10.60</td>
</tr>
<tr>
<td>BN-NPB</td>
<td>-7.51</td>
<td>-13.41 to -0.98</td>
</tr>
<tr>
<td>BN-NBUD</td>
<td>2.52</td>
<td>-2.14 to 7.41</td>
</tr>
<tr>
<td>NDI</td>
<td>-0.86</td>
<td>-1.86 to 0.16</td>
</tr>
<tr>
<td>Necrosis</td>
<td>-3.79</td>
<td>-8.04 to 0.67</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>-3.72</td>
<td>-8.93 to 1.79</td>
</tr>
</tbody>
</table>

Results are expressed as % change in marker frequency with a doubling in UV exposure or with a doubling in serum 25(OH)D levels.

### Table II. Linear regression analyses of log-transformed CBMN-cyt assay biomarker frequencies on log total serum 25(OH)D in all participants and on log sun exposure in categories of participants with serum 25(OH)D below and at or above >50 nmol/l

<table>
<thead>
<tr>
<th>CBMN-cyt assay biomarker</th>
<th>Solar UV exposure</th>
<th>Pinteraction b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D &lt; 50 nmol/l (n = 21)</td>
<td>25(OH)D ≥ 50 nmol/l (n = 187)</td>
</tr>
<tr>
<td></td>
<td>% Change</td>
<td>95% CI</td>
</tr>
<tr>
<td>BN-MN</td>
<td>7.24</td>
<td>2.01 to 12.75</td>
</tr>
<tr>
<td>BN-NPB</td>
<td>-8.56</td>
<td>-14.86 to -1.81</td>
</tr>
<tr>
<td>BN-NBUD</td>
<td>2.05</td>
<td>-2.88 to 7.23</td>
</tr>
<tr>
<td>NDI</td>
<td>-1.49</td>
<td>-2.53 to -0.43</td>
</tr>
<tr>
<td>Necrosis</td>
<td>-4.79</td>
<td>-9.23 to -0.13</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>-4.57</td>
<td>-10.04 to 1.24</td>
</tr>
</tbody>
</table>

Results are expressed as percent change in marker frequency with a doubling in sun exposure.

aAdjusted for age, sex, BMI and alcohol.
bInteraction between solar UV exposure and serum 25(OH) levels above and below 50 nmol/l.

### Discussion

We assessed the associations of personal solar UV exposure with DNA damage and cytotoxicity using the CBMN-cyt assay. There was some evidence of an increase in chromosomal breakage or loss (as indicated by increased frequency of BN-MN) and suppression of mitogenesis (reduced NDI) with increasing solar UV exposure. It has previously been shown that formation of UV-induced DNA base damage, CPDs, is positively correlated with micronuclear formation; there is also a wealth of evidence that chemical and physical genotoxins that induce base damage can also induce micronuclei (10,17–20). Incomplete repair of these DNA lesions may result in the formation of transient abasic sites and DNA strand breaks or stalled replication, which may result in DNA strand breaks leading to acentric chromosome fragment formation and micronuclei (10). There was also weak evidence of falls in formation of dicentric chromosomes (as indicated by BN-NPB frequency) and lymphocyte necrosis with increasing solar UV. Although levels of serum 25(OH)D showed no association with DNA damage, it appeared that serum 25(OH)D levels may modify the observed associations of solar UV with micronuclear formation and suppression of mitogenesis both being weaker when 25(OH)D levels were sufficient.

Our finding that frequency of DNA damage increased with increasing solar UV exposure in circulating lymphocytes is consistent with several previous reports (7–9,21), which have demonstrated associations between solar UV exposure and DNA damage in skin cells (21) and in circulating lymphocytes (9); increased levels of lymphocytic DNA damage were observed in summer (7,8). Balansky et al. (2003) have also
demonstrated a regulation in internal cells, following exposure of skin to UV irradiation, where increased formation of bulky DNA adducts in both lung and bone marrow and induction of cytogenetic damage in bone marrow and peripheral blood erythocytes was observed (22). This induction of UV-induced DNA damage in non-exposed tissue observed with sun exposure in the period closest to the time when blood was collected (7–9, 22) is consistent with our observation of DNA damage in lymphocytes associated with average sun exposure in the preceding 6 weeks. Adjustment for BMI and alcohol intake did not weaken but rather slightly strengthened the associations we observed between sun exposure and DNA damage; all participants were non-smokers. An increase in MN, induced by DNA strand break events, is usually accompanied by formation of dicentric chromosomes and nucleoplasmic bridges because they, and the associatedacentric chromosome fragments from which MN may originate, are formed as a result of DNA strand break misrepair (23). Therefore, the reduction in BN-NPB with increased UV dose that we observed seems counter-intuitive unless, perhaps, exposure to low dose UV in some way protects against DNA strand break misrepair or telomere end-fusion, from which BN-NPEI are also thought to originate, although without generation of accompanying MN (14,23). That there may be a unique response in the telomeres to UV exposure is suggested by the recent observation that UV-induced pyrimidine dimers in the TTAGGG telomere repeat sequence are not repaired (24), possibly to prevent generation of transient abasic sites and strand breaks which may destabilise telomere structure and function or cause excessive shortening of the telomere.

The NDI or frequency of binucleated cells is a robust measure of mitogen response, which is a conventional bio-marker of immune function (25,26). Although we do not have data on white blood cell counts or white blood cell differential counts, it was previously shown that a reduced NDI, as measured by calculating % binucleated cells in the CBMN assay, and an increased micronucleus count are significantly associated with reduced white blood cell counts (25–27). Moreover, reduction in NDI has been linked to UV-induced immune suppression, where a decreased lymphocytic response to mitogenic stimulation was observed following UV irradiation (28,29). The weaker mitogenic response (reduced NDI) in circulating lymphocytes, with increasing solar UV exposure, is suggestive of immune suppression. Additionally, the ability for cutaneous UV exposure to cause immune suppression at locations remote from the site of exposure suggests that this association is systemically regulated (30). Thus, a slight alteration in the function of lymphocytes, which is central in maintaining a normal immune function, may have long-term effects with respect to health outcomes such as cancer (22,31,32).

Since solar UV exposure initiates epidermal production of vitamin D, it may be of interest that solar UV exposure appeared to increase BN-MN frequency and reduce NDI more when serum 25(OH)D was at levels generally considered to be insufficient (<50 nmol/l) than when it was sufficient (33). Higher levels of vitamin D may mitigate against UV-induced suppression of mitogen response and therefore reduce NDI by reducing DNA damage events such as micronucleus formation that cause mitotic delay (32) or by stimulating T-cell division as was shown in haemodialysis patients treated with calcitriol (34). In vivo and in vitro studies have shown that vitamin D can prevent DNA damage directly or indirectly by inducing cell cycle arrest and increasing the activity of DNA repair (35–41). A clinical trial on colorectal carcinoma patients showed daily treatment with 800 IU of vitamin D reduced levels of 8-hydroxy-2′-deoxyguanosine, a marker for DNA damage, in biopsies taken from the colorectal crypts (42). The association between 25(OH)D levels and apoptotic activity has previously been shown, both in vitro and in one clinical trial. Apoptotic activity has also been linked to sun exposure where high sun exposure was associated with increased Bcl-2 translocation which is anti-apoptotic (43–45). We, however, did not observe any association between sun exposure or serum 25(OH)D and apoptosis. Dietary vitamin D intake was not measured in this study as its content in food is minimal in Australia (46).

Although 25(OH)D is used to determine vitamin D status, it is not the active metabolite; it is further converted to 1,25 dihydroxy vitamin D (1,25(OH)2D), its active metabolite. Depending on whether it acts through its genomic or rapid response pathway (30,31), 1,25(OH)2D is able to mimic UV-induced immune suppression (47) or to reduce UV-induced DNA damage and cell death (48,49) in skin. Although the preferences for one pathway over the other are not clearly defined, some evidence suggests it may relate to specific conformations of vitamin D analogues (48). Thus to further understand the possible effects of vitamin D in reducing DNA damage due to solar UV radiation may require a study that includes study of vitamin D conformations and locations of vitamin D other than 25(OH)D in serum (48–50).

The major strength of this study is the use of the CBMN-cyt assay in detecting DNA damage and cytotoxicity in peripheral blood lymphocytes, which is a validated and efficient technique for measuring chromosomal instability, cell death and cytostasis (14). In addition, the approach to recall of sun exposure over 16 weeks was based on a method used to obtain recall of lifetime sun exposure, which has been shown to have good reliability (51). This study is the first to concurrently examine the associations of solar UV exposure and serum 25(OH)D with DNA damage and cytotoxicity in lymphocytes. The study may have been strengthened by simultaneous measurement of UV-specific base damage or mutations in lymphocytes but, based on observations in other studies, these mutations are likely to be strongly correlated with the CBMN-cyt biomarkers (10,17,52).

Conclusion

Personal solar UV exposure is associated with DNA damage in peripheral blood lymphocytes and possibly with a reduction in lymphocytes’ responsiveness to mitogenic stimuli, an indicator of immune suppression. Although serum 25(OH)D concentration showed little evidence of an association with DNA damage and mitogen response, there was evidence that it modified the effect of solar UV exposure on both these biomarkers. The direction of this effect suggested that vitamin D sufficiency may be needed to prevent lymphocyte DNA damage from UV exposure.

Supplementary data

Supplementary Table 1 is available at Mutagenesis Online.

Funding

National Health and Medical Research Council of Australia (Project Grant ref 464895).
Vitamin D can prevent DNA damage directly or indirectly by calcitriol (34). In vivo when serum 25(OH)D was at levels generally considered to immune suppression, where a decreased lymphocytic response assay, and an increased micronucleus count are significantly counts, it was previously shown that a reduced NDI, as generation of accompanying MN (14,23). That there may be exposure to low dose UV in some way protects against DNA acentric chromosome fragments from which MN may damage; all participants were non-smokers. Intake did not weaken but rather slightly strengthened the DNA adducts in both lung and bone marrow and induction of demonstrated a regulation in internal cells, following exposure includes study of vitamin D conformations and locations of defined, some evidence suggests it may relate to specific DNA damage and cytotoxicity in lymphocytes. The study may Sun exposure initiates epidermal production of An increase in MN, induced by DNA strand break events, is et al. (Project Grant ref 464895). Funding this effect suggested that vitamin D sufficiency may be needed to mitogen response, there was evidence that it modified the effect immune suppression. Although serum 25(OH)D concentration Personal solar UV exposure is associated with DNA damage in observations in other studies, these mutations are likely to be UV-B induced DNA damage and cell death (48,49) in skin. Although the response pathway (30,31), 1,25(OH)2D is able to mimic UV-dihdyroxy vitamin D (1,25(OH)2D), its active metabolite. We, however, did not observe any association between sun exposure where high sun exposure was associated with clinical trial. Apoptotic activity has also been linked to sun exposure, which has been shown to have good reliability and in one patients showed daily treatment with 800 IU of vitamin Carolin Salisbury and Theodora Almond are acknowledged for their excellent Funding.