The association between personal sun exposure, serum vitamin D and global methylation in human lymphocytes in a population of healthy adults in South Australia

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Introduction

This study highlights the role of solar UV exposure in influencing the biological mechanisms, in not just exposed sites, but in also, unexposed sites. This is based on our previous observations showing:

(i) A positive link between solar UV exposure and prostate cancer risk (Nair-Shalliker el al, 2012, 2013)

(ii) A positive association between solar UV exposure and lymphocytic DNA damage, such as micronuclei (MN) formation (Nair-Shalliker el al, 2012).

MN formation, which is a biomarker for chromosomal breakage or loss, can also result from global demethylation (GM) of DNA repeat sequences, such as long interspersed nucleotide element-1 (LINE-1). Exposure to ionising UV radiation in mouse skin can result in LINE-1 demethylation or induction of MN (4, Aypar et al., 2012). No studies have yet shown a link between solar UV exposure and GM in human lymphocytes, i.e. in unexposed sites.

Aim

- Evaluate the relationship between solar UV exposure and GM pattern in LINE-1 repetitive elements, in peripheral blood lymphocytes, from healthy adults.
- Examine the confounding effects of serum 25(OH)D,
- Examine the link between lymphocytic LINE-1 methylation and DNA damage.

Materials and Methods

Study population: 208 healthy male and female volunteers from Adelaide, South Australia, who were aged between 25 and 60 years.

Ineligibility criteria:

- current cigarette smokers or had smoked within the past six months
- receiving medical treatment for any life-threatening disease or had received it within the past six months
- women who were pregnant or planning a pregnancy
- taking daily dietary supplements exceeding 50% of the recommended daily allowance.

Data collection and analysis

- Baseline questionnaire was used to obtain social and demographic factors, sun sensitivity, and sun protection behaviour, and recall time spent outdoors in the preceding 16 weeks.
- Blood was collected in heparin tubes, processed within 24 hours and assayed for:
  - Serum vitamin D (25OHD) using enzyme immunoassay (BEST 2000 Analyser).
  - LINE-1 methylation using genomic DNA isolated from lymphocytes using DNeasy Blood and Tissue kit (Qiagen, Australia).
  - Lymphocytic DNA damage using cytokinesis-block micronucleus cytome assay (CBMN).
- Personal solar UV exposure was calculated from recalled hours of weekly exposure over the preceding six weeks before blood collection and weighted against ambient UV irradiance between 9am and 5pm at their location in that week (Nair-Shalliker et al., 2012).

Statistical analysis

- Log transformed GM as dependent variable and weekly solar UV exposure and serum 25(OH)D as independent variables.
- Linear regression was used to examine the association between the dependent and independent variables, using the percent change in marker frequency with a doubling in either solar UV exposure or 25(OH)D, adjusting for age and sex.

Results

Table 1. Linear regression analyses of log transformed LINE-1 methylation on (i) log solar UV exposure and (ii) on log 25(OH)D levels in serum, adjusting for age and sex.

Variable	Global methylation	
	% change	*p-value*
Solar UV exposure (mJ/cm^2)	**-0.5**	**0.0003**
25(OH)D (all participants)	0.1	0.7
25(OH)D (<50 nmol/L)[a]	0.9	0.6
25(OH) (≥50 nmol/L)[a]	0.03	0.9

[a]p-interaction =0.55 for the association between solar UV exposure and serum 25(OH) levels above and below 50nmol/L.

- A 0.5% change in LINE-1 DNA methylation may seem small, but it may nevertheless be biologically significant.

- A study in school-age children showed that plasma retinol and C-reactive protein are inversely associated with LINE-1 methylation with effect sizes in the range of 0.15-0.30% (Perng et al., 2012), thus suggesting that changes in LINE-1 methylation of this order of magnitude could be biologically relevant.
- No association between GM and MN formation suggests that changes in LINE-1 methylation alone do not adequately explain MN induction.
- The positive link between GM and NPB formation are consistent with our previous observation of an inverse relationship between solar UV exposure and NPB formation.

Table 2. Linear regression analyses of log transformed LINE-1 methylation on frequencies of log transformed CBMN assay biomarkers, adjusting for age and sex.

Discussion

CBMN-cyt assay biomarker	% change	p-value
Micronuclei (MN)	-0.2	0.9
Nucleoplasmic bridges (NPB)	**4.3**	**0.02**
Nucleoplasmic buds	-0.2	0.9
Nuclear division index	-0.3	0.3
Necrosis	**4.3**	**0.0005**
Apoptosis	1.2	0.4

- UVB skin carcinogenesis is characterised by an abundance of cytosine (C) > thymidine (T) transitions at dipyrimidine sequences. Cytosine is also present in a methylated form, 5-methylcytosine, which has ~5-15 fold higher energy absorption in the UVB range (295-320nm) than cytosine (Greinert, et al., 2012). This higher absorption and greater UVB-induced CPD formation can lead to greater loss of 5-methylcytosine than the unmethylated cytosine.
- 5-methyl-tetrahydrofolate (5MTHF), a primary methyl donor for DNA methylation, is UVB sensitive; its depletion can lead to DNA hypomethylation (Rochette and Brash, 2010). Thus the link between sun exposure and LINE-1 hypomethylation might be mediated by UV-induced 5-MTHF depletion.
- UV-induced sub-telomere demethylation of CPDs could indirectly induce mechanisms that elongate telomeres and reduce the occurrence of NPBs (8).
- UV-induced telomerase activity may give rise to a pro-inflammatory state leading to increased necrosis (Ghosh et al., 2012).

Conclusion

There may be a role for sun exposure and global methylation in influencing disease processes, in unexposed sites, and this warrants further investigation.

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