Markers of HIV-1 infection and its pathogenesis
Maas, J.J.

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Chapter 7

Amsterdam Cohort Study among HIV infected homosexual men: no indications for an ultraviolet B radiation induced decline of immunological parameters after sunlight exposure


Submitted
Abstract

Introduction Whether sunlight (especially ultraviolet B radiation: UVB) exposure is harmful to the cellular immunity of HIV-1 infected individuals and whether it is associated with an accelerated progression toward AIDS is still under debate. Study and methods Among HIV+ homosexual participants of the Amsterdam Cohort Study, the degree of exposure to UVB was, assessed over a period from 1995 until 1997 by means of a retrospective questionnaire. Two UVB measures were calculated: a cumulative measure (the total amount of UVB exposure received over two years) and a short-term measure (the amount of UVB exposure per stated period). Using both the nonparametric Mann-Whitney test and robust regression, the association between the total amount of UVB received in the two-year interval and the slopes of the following immunological parameters were studied: CD4+ T-cell count, CD4/CD8 ratio and T cell reactivity after stimulation with CD3 antibodies. Secondly, the short-term association between UVB exposure per period and the immunological parameters were studied with linear mixed-effect models (LME). Results In the study group with a median CD4+ T-cell count of 480 $\times$ 10^6/l, no association could be established between UVB exposure, and the immunological parameters. Conclusion Among HIV-1 infected individuals, no indications could be found between either the cumulative or short-term UVB exposure and markers of cellular immunity.
**Introduction**

Despite the great number of publications on the harmful effects of Ultraviolet Radiation-B (UVB), as is present in sunlight, on the cellular immunity in HIV-1-infected subjects, this issue is still a matter of debate. It is evident from *ex vivo* *in vitro* research that UVB has a negative effect on the number and function of peripheral T cells, Langerhans cells \(^1\)\(^-\)\(^4\) and contact hypersensitivity response \(^5\), and that UVB is capable of HIV reactivation *in vitro* \(^6\). An important indication that UVB suppresses human immune responsiveness was the finding that exposure of human skin to sub-erythemal doses of UVB followed by the application of a contact sensitiser to the irradiated site results in a decreased hypersensitivity (CHS) response \(^7\). It was shown that such suppression was also induced when whole body radiation was applied, even though the site of application of the contact sensitiser was shielded from UVB radiation. This is an indication that the effects of UVB on the skin immune system do not stay confined locally. It was demonstrated that skin type has no influence on potential of UVB. Black volunteers were as equally susceptible as Caucasians to UVB-induced suppression of CHS \(^8\).

Furthermore, because a decrease in the CD4\(^+\) T-cell count and CD4/CD8-ratio has been described among healthy subjects after non-experimental UVB exposure \(^9\), we hypothesised that HIV-1 infected subjects are possibly more vulnerable to the immuno-suppressive effects of UVB \(^10\). The aim of this uniquely designed study therefore was to investigate the association between non-experimental and day-to-day UVB exposure and the cellular immunity of HIV-1-infected subjects. For the assessment of the exposure to UVB, we designed a two-year retrospective questionnaire. This questionnaire enabled us to estimate both short-term and cumulative exposure to UVB. Subsequently, we investigated whether there was an association between the calculated exposure to UVB and the immunological assessments determined in the two-year time interval.
Material and methods

Study group
Since October 1984 HIV seronegative and seropositive homosexual men have been enrolled in the Amsterdam Cohort study on the natural history of HIV and AIDS. All HIV-1 seropositive participants were seen at three monthly visits, at which a medical history was taken, a physical examination was done and blood was drawn for various virological and immunological tests.

Exposure assessment
In the period from January until April 1997, questionnaires were handed out to all participants who visited our study site. The self-administered questionnaire was designed to assess the exposure of sunlight and artificial UVB sources in the two preceding years. A more detailed explanation of the exposure assessment used is described elsewhere.
Sunlight exposure was expressed as the daily number of hours outdoors reported during: holiday, occupational, and leisure associated activities. In addition, self reported data on the coverage by clothing, the sort of activities (e.g. swimming, sailing), the countries of destination and the season enabled us to weight each reported hour for the effects of these factors on the UVB dose received. The weighting factors for the effects of clothing and activity were derived from literature. Weighting factors for the effect of latitude and season were calculated on the basis of data on environmental irradiiances obtained from the Laboratory of Radiation Research of the RIVM. Exposure due to the use of a solarium was estimated by assuming that each visit to the solarium is equivalent to one MED (=200J/m² at wavelength 297 nm).

The data from the questionnaire enabled us to assess the exposure to UVB both cumulative - over the two preceding years - and separately for different periods such as holiday periods in sunny countries. This latter UVB measure is especially useful in studying the short-term effects of UVB on the immune system and the possible mechanisms of adaptation. Because the impact of sunscreen use and skin type on the cellular immunity is
HIV and UVB exposure

questionable, they were not included in the exposure assessment. Subsequently, we examined whether there was an association between those UVB measures and CD4⁺ T-cell count, CD4/CD8 ratio and T-cell reactivity after stimulation with CD3 antibodies (CD3 MAb). CD3 MAb is known to be a sensitive marker that can be impaired early in the HIV-1 infection course.

Statistics

Different analyses were performed using categorical as well as continuous UVB data. With the least square linear regression technique, individual slopes were calculated using the data of the immunological assessments gathered in the two-year time interval. The Mann-Whitney-U-test was used to compare the individual slopes of subjects with a low versus high cumulative UVB exposure (≤ 75 percentile). Secondly, a robust M-estimates regression analysis was performed. This technique can be used if the data do not satisfy the normality conditions or if the data contain significant outliers. In this analysis, we examined whether there was a linear correlation between the continuous cumulative UVB measures and the individual slopes of the immunological assessments. Subjects who had started with Highly Active AntiRetroviral Treatment (HAART) were excluded from those two analyses. In the third analysis, by means of a linear mixed-effect model (LME) for longitudinal data, the short-term UVB effect on the immunological parameters was examined.

In the baseline model, we first investigated the association between the immunological parameters and calendar time. In an extended model, the short-term cumulative UVB exposure per period was added. The effect of UVB exposure on the markers of cellular immunity was evaluated by examining whether there was an association between the short-term cumulative UVB exposure received during periods of one week, two weeks or three months prior to the immunological assessment, and the outcome of the immunological test. In the case of a suppressive UVB effect a negative deviation is seen of the quartile intercept, compared with the baseline intercept (Figure 1). In the case of a beneficial effect, a positive deviation is seen.
Markers of HIV-1-infection and its pathogenesis

Figure 1.

Schematic representation of the used mixed effects model

The short-term cumulative UVB exposure received, for example, three months before an immunological assessment was divided in four equal parts according to the quartiles, with the lowest first quartile as reference. The times of the immunological assessments are represented by the dotted lines. Additionally, because the short-term cumulative exposure is defined as the total UVB exposure received during, for example, three months, prior to the immunological assessment, those dotted lines also represent the different UVB quartiles.

The bold line represents the development of the immunological parameter over calendar time for a person belonging always to the lowest exposure category. The first quartile is not presented because it is used as a reference.

Subsequently, we examined whether UVB exposure was associated with a change of intercept. In other words, whether UVB exposure was capable of causing a temporary suppression of the immunological marker (a negative deviation of the intercept from the baseline model) or a positive effect (i.e. a positive deviation from intercept). Depending on the individual number of immunological assessments, persons were able to switch between the different quartiles more than once.
HIV and UVB exposure

Furthermore, in this extended model, UVB exposure was categorised in quartiles. HAART usage was included in the model as a time-dependent co-variable.

Laboratory Methods

All serological tests were carried out in fresh sera. T-cell subsets were tested using fresh cells. CD4\(^+\) and CD8\(^+\) T-cells were enumerated by a direct immunofluorescent technique using monoclonal antibodies and a flowcytometric system. To measure T cell reactivity, fresh cells were stimulated in a whole culture system with CD3 MAb. Reactivity was expressed as the percentage of median responses detected in concurrently running cultures of five healthy controls.

Results

Baseline characteristics of UVB exposure

Of the 114 questionnaires handed out to HIV-1 positive homosexual men in the period January 1997 until May 1997, 67 were returned (58%). One subject was excluded from the analysis because he returned the questionnaire empty. Of the remaining 66 HIV-1 infected asymptomatic subjects, nine had recently started with HAART. The median CD4\(^+\) T cell count, determined at the beginning of the questionnaire period, was 480 x 10\(^6\)/l (inter quartile range (IQR): 320-613). The median number of visits and consequently the number of serial immunological determinations was 8 (IQR: 6-12), with a median study interval of 2.0 years (IQR: 1.4-2.0). The median number of weighted hours of UVB exposure over two years was 1052 hours (IQR: 537-1803) (Figure 2) or 1.4 hours per day (IQR: 0.7-2.5). Many of the participants reported holiday periods in southern sunny countries. Seasonal fluctuations also showed up very clearly in the answers to the questionnaire (data not shown). By January 1998, one year after completing the questionnaire, only one person out of the 57 untreated HIV-1 infected individuals in our study had developed AIDS (Candida oesophagitis).

Results using the cumulative UVB measure

In none of the two analyses using the cumulative UVB measure could an association be found
between UVB exposure and the individual slopes of the immunological markers (table 1).

Results using the short-term UVB measure
In the two years preceding the finalisation of the questionnaire, 573 immunological assessments were carried out. In the baseline model, containing only the immunological markers and calendar time, both CD4+ T cells and T cell reactivity gradually decreased over calendar time (-12.6 cell x 10⁶/l/year and -0.1%/year, respectively), whereas CD4/CD8 ratio very slowly increased (1%/year). Although indications were found in most of the UVB quartile analyses of a negative deviation from the baseline model without UVB, none of these findings were significant. In all the analyses, however, a significant relation was found between the immunological parameters and HAART usage. In table 2 only the data from the analysis of the three monthly period are shown.
HIV and UVB exposure

Figure 2.
Frequency distribution of the cumulative numbers of outdoor hours in the two years preceding the completion of the questionnaire filled in by 57 HIV-1 infected homosexual men, after weighting for activity, clothing, latitude and season.
Markers of HIV-1-infection and its pathogenesis

Table 1.
Results of the non-parametric and robust linear model, using the cumulative UVB measure obtained from 57 HIV-1 infected individuals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non parametric test</th>
<th>Robust linear model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P value</td>
<td>slope</td>
</tr>
<tr>
<td>CD4+</td>
<td>0.89</td>
<td>-0.05*</td>
</tr>
<tr>
<td>CD4/CD8</td>
<td>0.19</td>
<td>0.001*</td>
</tr>
<tr>
<td>ACD3</td>
<td>0.59</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*95% confidence interval, † unit:100 cells/year, ‡ unit: %/year, * unit: %/year

Table 2.
Results longitudinal analysis, with the degree of CD4⁺ T cell, CD4/CD8 ratio, and T cell reactivity change, determined in the three months prior to the immunological assessment, per quartile UVB exposure

<table>
<thead>
<tr>
<th>Interval immunological assessment and time exposure: three months</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVB exposure (number of hours after weighting)</td>
</tr>
<tr>
<td>CD4⁺ T cell count [deviation] [p value (1)]</td>
</tr>
<tr>
<td>UVB quartiles</td>
</tr>
<tr>
<td>0.5-13.6 hours</td>
</tr>
<tr>
<td>13.6-54.3 hours</td>
</tr>
<tr>
<td>54.3-795 hours</td>
</tr>
</tbody>
</table>

CD4 cells, fourth root transformed, CD4/8 ratio, third root transformed, T cell reactivity after stimulation with CD3 antibodies (fifth root transformed), deviation of the intercept from baseline, overall statistics 0.39 overall statistics 0.22, overall statistics 0.33, UVB exposure divided in four UVB exposure quartiles, with the lowest quartile as reference, unit: transformed immunological marker per year, HAART: Highly Active AntiRetrovirale Therapy.
**HIV and UVB exposure**

**Discussion**

In the Western World, a tanned skin is considered as a sign of good health and prosperity. This, however, is only one side of the coin. Due to the change in sun seeking behaviour in the Western World, a sharp rise in the incidence of UVB associated skin cancers such as (non) melanomas, is seen. In addition, in a great number of *ex vivo* *in vitro* and animal studies harmful UVB effects are described in the various compartments of the immune system. In our study however, we primarily focussed on asymptomatic HIV-1 infected individuals to determine whether there were indications of UVB induced immunological impairments. Additionally, we investigated whether this influenced the progression rate to clinical AIDS. In this present study, no significant indications were found for UVB associated immune modulation and HIV activation.

There is a discrepancy between the results found in the (*ex vivo* *in vitro* animal and clinical literature. In contrast to clinical studies, in a great number of *in vitro* and animal studies clear indications were found for an UVB associated immune modulated effect and HIV activation. In a recent review of Akaraphanh et al., however, which described the results of eleven clinical UVB studies among HIV-1 infected individuals, an UVB associated CD4+ T cell decline could be established in one study only.

By assessing individual exposure in this way, different sources of inaccuracy may have been introduced. First, the exposure assessment based on a retrospective questionnaire relies heavily on the recall and reporting of the participant, thereby always introducing a bias. On the other hand, it is known that the highest exposure per day is received during holidays. Assuming that it is easier to have a more accurate remembrance of a special and joyful event such as a holiday, both with respect to geographical location and season, the questionnaire can be considered sufficient for identification of short periods with high exposure. Finally, it must be emphasised that due to the small sample size, subtle UVB effect can have been missed.
Markers of HIV-1-infection and its pathogenesis

In summary, although we were capable of detecting periods of high UVB exposure and seasonal fluctuations accurately, in agreement with a clinical study of by Saah et al.\textsuperscript{30}, in neither of the analyses we were able to detect clinical relevant short-term adverse UVB effects among HIV-1 infected homosexual individuals.

Acknowledgements
This study was performed as part of the Amsterdam Cohort studies on HIV-1 infection and AIDS, a collaboration between the Municipal Health Service, The Academic Medical Center, the CLB, Sanguin Blood Supply Foundation and Laboratory for Experimental and Clinical Immunology, Amsterdam, The Netherlands. We thank Dr. H. Slaper and Dr. J. Matthijsen from the Laboratory of Radiation Research of the RIVM for their assistance, Dr. René Keet for his assistance with the design of the study, Gillian Cowie for her editorial support and Nel Albrecht and Marja Dekker for the daily organisation of the cohort study. Most of all we are indebted to the men in the cohort for participating so willingly for so long.
HIV and UVB exposure

Reference List


HIV and UVB exposure


