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**No indications for an ultraviolet B radiation
induced decline of immunological parameters after
sunlight exposure among HIV infected homosexual
men**

J. Maas, F. Termorshuizen, R.B. Geskus,
R.A. Coutinho and H. van Loveren

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Samenvatting

Het is nog onduidelijk of zonlicht, en in het bijzonder ultraviolet B, schadelijk is voor cellulaire immuniteit in personen die met HIV zijn geïnfecteerd, leidend tot een versnelde progressie van AIDS. Wij hebben bij HIV+ homoseksuele deelnemers in de “Amsterdam Cohort Study” de mate van UVB blootstelling vastgesteld gedurende de periode van 1995 tot 1997, door middel van een retrospectieve questionnaire. Een tweetal maten werd daarbij gehanteerd: een cumulatieve maat (het totaal aan UVB opgelopen gedurende de gehele periode) en een korte termijn maat (de blootstelling gedurende het aangegeven tijdvak). Gebruikmakend van de non-parametrische Mann-Whitney test en regressie analyse werd de associatie tussen de totale UVB blootstelling die werd opgelopen gedurende twee jaar en het verloop van de volgende immunologische parameters bestudeerd: $CD4^+$ T cel aantallen, CD4/CD8 ratio, en T cel reactiviteit na stimulatie met anti-CD3 antilichamen. Voorts werd de korte termijn associatie tussen UVB blootstelling per periode en de immunologische parameters bestudeerd, gebruikmakend van een lineair mixed-effect model (LME). Met geen van beide benaderingen was het mogelijk een significant effect van blootstelling op deze parameters aan te tonen. In de benadering met de korte termijn blootstelling was een trendmatig effect te zien. Het is niet aannemelijk dat dit geringe effect klinische relevantie voor de individuen in het onderzoekscohort heeft, maar het geeft wel aan dat er mogelijk een effect van UV in een grotere onderzoeksgroep vastgesteld zou kunnen worden.

Summary

Whether sunlight (especially ultraviolet B radiation: UVB) exposure is harmful to the cellular immunity of HIV infected individuals and whether it is associated with an accelerated progression toward AIDS is still under debate. 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). 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. Among HIV infected individuals, no indications could be found between the two year cumulative UVB exposure and markers of cellular immunity. Using the linear mixed effects model for longitudinal data, a slight trend in lower values of markers of cellular immunity after higher short term UVB-exposure was noted. It is quite unlikely that this small effect has clinical significance for the individuals of the study cohort, yet it may indicate that an effect of UV might have been established in a larger cohort.

1. 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-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 sensitizer 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 sensitizer 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 effects. 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-infected subjects are possibly more vulnerable to the immuno-suppressive effects of UVB [10]. This is especially relevant because it is nowadays possible to treat Kaposi's sarcoma with photo-dynamic therapy [11].

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-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.

2. Material and methods

2.1 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 [12]. All HIV 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.

2.2 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 [13]. A more detailed explanation of the exposure assessment used is described elsewhere [14]. 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 irradiances obtained from the Laboratory of Radiation Research of the RIVM [15-17]. Exposure due to the use of a solarium was estimated by assuming that each visit to the solarium is equivalent to one MED ($=200\text{J/m}^2$ at wavelength 297 nm) [18].

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 questionable, they were not included in the exposure assessment [8,19]. 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 [20,21].

2.3 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 [22]. 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 [23]. 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. Furthermore, in this extended model, UVB exposure was categorised in quartiles. HAART usage was included in the model as a time-dependent co-variable.

2.4 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.

3. Results

3.1 Baseline characteristics of UVB exposure

Of the 114 questionnaires handed out to HIV 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 infected asymptomatic subjects, nine had recently started with HAART. The median CD4⁺ T cell count, determined at the beginning of the questionnaire period, was $480 \times 10^6/l$ (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) 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 infected individuals in our study had developed AIDS (Candida oesophagitis).

3.2 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 1a).

3.3 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 ($-12.6 \text{ cells} \times 10^6/l/\text{year}$ 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 1b only the data from the analysis of the three monthly periods are shown.

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

Parameters	Non parametric test	Robust linear model		
	p value	Slope	95%CI [#]	p value
CD4 ⁺	0.89	-0.05 [†]	-1.17 to 1.06	0.93
CD4/CD8	0.19	0.001 [‡]	-0.004 to 0.006	0.66
ACD3	0.59	0.003 [*]	-0.008 to 0.01	0.61

[#] 95% confidence interval, [†] unit: 100 cells/year, [‡] unit: %/year, ^{*} unit: %/year

Table 1b. 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.

<i>1.1.1.1 Interval immunological assessment and time exposure: three months</i>						
UVB quartiles	UVB exposure (number of hours after weighting)					
	CD4 ⁺ T cell count [†] deviation [#]	p value ⁽¹⁾	CD4/8 ratio [‡] deviation [#]	p value ⁽²⁾	T cell reactivity* Deviation [#]	p value ⁽³⁾
0-0.5 hours	0	-	0	-	0	-
0.5-13.6 hours	0.009	0.44	-0.011	0.55	0.022	0.45
13.6-54.3 hours	-0.008	0.19	-0.004	0.68	-0.021	0.20
54.3-795 hours	-0.005	0.18	-0.010	0.11	-0.010	0.33
HAART [@]	0.03	0.06	0.06	0.03	0.08	0.05

[†] 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, ⁽¹⁾ overall statistics 0.39 ⁽²⁾ overall statistics 0.22, ⁽³⁾ 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 Treatment.

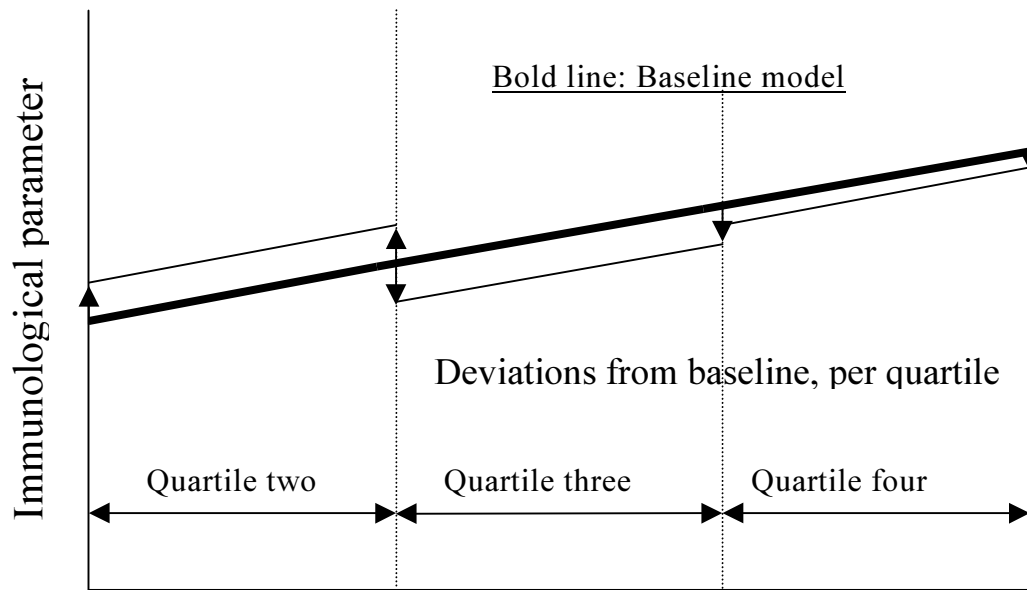


Figure 1. Schematic representation of the used mixed effects model.

Calendar time

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.

4. Discussion

In the Western World, a tanned skin is considered as a sign of good health and prosperity [24]. 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 [25-27]. 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 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. Among HIV infected individuals, no indications could be found between the two year cumulative UVB exposure and markers of cellular immunity. Using the linear mixed effects model for longitudinal data, a slight trend in lower values of markers of cellular immunity after higher short term UVB-exposure was noted.

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 Akaraphanich et al. [28], however, which described the results of eleven clinical UVB studies among HIV infected individuals, an UVB associated CD4⁺ T cell decline could be established in one study only [29].

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 [30]. Finally, it must be emphasized that due to the small sample size, subtle UVB effect can have been missed.

In summary, although we were capable of detecting periods of high UVB exposure accurately, in agreement with a clinical study of by Saah et al. [31], in neither of the analyses we were able to detect statistically significant effects on markers of cellular immunity among HIV infected homosexual individuals. In the repeated measurement analysis a slight trend towards lower values of markers of cellular immunity after short term UVB-exposure was noted. It is quite unlikely that such a small effect has clinical significance for the individuals of the study cohort, yet it may indicate that a subtle effect of ambient UVB exists. Confirmation of this notion requires a larger cohort. We are currently investigating seasonal influences on markers of cellular immunity in the entire cohort of HIV positive individuals, from which the participants of the study described here were recruited.

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Appendix 1 Mailing list

1. Dr.Ir. M.W.J. Wolfs, algemeen directeur Keuringsdienst van Waren
2. Dr. J.J. Ende, Keuringsdienst van Waren
3. Voorzitter van de Gezondheidsraad
4. Prof.Dr. J.C. van der Leun, Universiteit Utrecht
5. Dr.F.R. de Gruijl, Universiteit Utrecht
6. Prof.Dr. B.J. Vermeer, Universiteit Leiden
7. Depot Nederlandse Publikaties en Nederlandse Bibliografie
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9. Prof.Dr.Ir. D. Kromhout, directeur sector 2
10. Dr.Ir. G. de Mik, directeur sector 3/4
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12. Prof.Dr. J.G. Vos, hoofd LPI
13. Dr. R.C.G.M. Smetsers, hoofd LSO
14. Dr. B.J. Heij, NOP
15. Dr. W. Verwij, NOP
16. Dr. H. Slaper, LSO
17. Drs. J.J. Smit, CZE
18. Mw. Dr. A.H. Wijga, CZE
19. Ir. D.J.M. Houthuijs, LBM
20. Ir. P.H. Fischer, LBM
21. Dr. J. Maas, auteur
22. Drs. F. Termorshuizen, auteur
23. Dr. R.B. Geskus, auteur
24. Prof. Dr. R.A. Coutinho, auteur
25. Dr. H. van Loveren, auteur
26. SBD/Voorlichting & Public Relations
27. Bureau Rapportenregistratie
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- 43-47. Reserve exemplaren