Urinary Thymidine Dimer as a Marker of Total Body Burden of UV-Inflicted DNA Damage in Humans

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Abstract

High levels of DNA damage are induced in human skin following exposure to UV radiation. Cyclobutane thymidine dimer (T = T) is the most common of these lesions, which are enzymatically removed as oligonucleotides from DNA and further degraded before excretion in urine. Analysis of such repair products in the urine could serve as a biomarker of total body burden of UV exposure. The aim of this study was to examine the kinetics of T = T excretion following a single tanning session in a commercial solarium and to validate the method by delivering different doses. Ten individuals used the solarium for a total of 35 sessions of body tanning. Urine was collected before UV exposure and daily thereafter (up to 5 or 11 days) and T = T was analyzed using a very sensitive and quantitative ^{32}P-postlabeling technique combined with high-performance liquid chromatography. Following exposure, T = T levels increased dramatically and reached a peak 3 days later; afterwards, the T = T levels gradually decreased. The total amount of T = T excreted differed about 5-fold among subjects given an equal dose. A 50% excretion time was calculated using the excretion data for the first 5 days and it was found to be between 55 and 76 hours for different individuals. There was a good correlation between the amount of T = T excreted during days 1 to 5 and the delivered UV dose. Reducing exposure time to 50% lowered the amount of T = T to 47%; if half of the lamps were covered, T = T decreased to 44%. Our data show that urinary T = T could be a suitable noninvasive biomarker for UV exposure; a finding which could also be applicable to studies in children. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2868–72)

Introduction

The incidences of cutaneous malignant melanoma and other skin cancers have increased markedly in many countries of fair-skinned populations, such as Australia, New Zealand, North America, and northern Europe (1). In Norway and Sweden, for example, the rates of melanoma have more than tripled since 1958 to 1962 (2). One of the most important factors behind these trends is a changed life-style of outdoor activities, sun-intensive vacations, and indoor tanning (3).

UV radiation, particularly UVB, damages DNA in exposed cells and it is believed that UV-induced DNA lesions are important for the development of skin cancers (4). Strong support for this hypothesis comes from the observation of very high cancer risks in sun-exposed skin of persons suffering from the rare skin disease xeroderma pigmentosum (5). The main reason for their sensitivity is that these individuals have deficiencies in nucleotide excision repair (6), the main system removing DNA lesions formed by UV radiation (7). UV radiation causes many different types of DNA damage, including cyclobutane thymidine dimer (T = T; ref. 8). The nucleotide excision repair system removes a 24- to 32-base DNA fragment containing such lesions (7). After being degraded by enzymatic processing, a substantial fraction of the excised T = T is excreted as such in the urine (9, 10).

Human exposure to UV radiation and the analysis of the ensuing DNA damage differ from most exposures to chemical carcinogens because: (*a*) controlled doses can be delivered without ethical problems, (*b*) UV-specific lesions are formed at very high levels (9), and (*c*) target tissue can be analyzed. However, taking skin biopsies involves a certain amount of discomfort for the participating individuals and larger studies are, for this reason, difficult to carry out. For the same reason, children could generally not be studied. Moreover, skin biopsies reflect DNA damage only in a small area of the body. Urinary excreted T = T could therefore serve as a more generally applicable biomarker of DNA damage from UV, reflecting the total body dose. We have previously conducted a pilot study on two sunbathing volunteers which showed that T = T could be detected in human urine after exposure to solar radiation (10). In the present study, we used the same quantitative and very sensitive ^{32}P-postlabeling method combined with high-performance liquid chromatography (HPLC) analysis. Volunteers were exposed to UV radiation in a commercial solarium. Two types of analysis were done. First, the kinetics of urinary excretion of T = T were established and then different exposure schemes were used for studying the relationship between UV dose and amount of urinary T = T.

Materials and Methods

Study Subjects. Ten volunteers of Caucasian origin (seven females and three males; 26-54 years old) were recruited, and informed consent was obtained from all participants. The study plan was approved by the local ethical committee of the Karolinska Institute (Dnr 246/03). All volunteers answered a questionnaire about age, sex, skin type, weight, and height. Judged from the answers, two skin types were represented (three of type II and seven of type III). The volunteers were asked not to expose a larger part of their body to the sun or to use a solarium 1 week before and during the time of sample collection. The study was carried out during the periods April to May and August to September.

UV Exposure and Sample Collection. The solarium used (Ultra Tan, Vimmerby, Sweden) had a total intensity of 0.213 W/m^2 measured by a Sola-Hazard portable spectroradiometer (4D Controls Ltd., Redruth, United Kingdom). Spectroradiometric analysis gave a range from 250 to 400 nm (λ_{max} 347 nm). The

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percentage of UVB emission was 1% with a spike at 314 nm. In total, 35 tanning sessions were done, divided into five different types of exposures: session A—whole body tanning for 20 to 30 minutes, depending on the individual sunlight sensitivity (corresponding to an erythema-weighted dose of 256-383 J/m^2); session B—half the time of session A; session C—exposure of half the body (upper half of the solarium was covered with a special dark fabric), but the same time as in session A. Sessions A and B were repeated once. Morning void urine samples were collected before UV exposure and every consecutive morning for 5 days. For six tanning sessions of six different individuals, urine was also collected in the mornings of days 7, 9, and 11 after irradiation. Samples were collected in 15 mL polyethylene tubes, immediately frozen and kept at −20°C until analysis. The time between the individual sessions was at least 1 month.

Sample Analysis. For the kinetics study, urine samples from each day were analyzed separately. For the dose study, the urine samples from days 1 to 5 were pooled, and only day 0 samples were analyzed separately. Urine samples were allowed to melt at room temperature and they were filtered through a 0.22 μm gauge filter. T = T was purified from 10 μL of urine using an HPLC system. The retention time of T = T was determined by a repetitive HPLC analysis of a T = T standard (prepared previously; ref. 10) and it was found to be 8.2 minutes. A fraction eluting between 7.3 and 9.1 minutes was collected, lyophilized, and redissolved in 40 μL of water. Pyrimidine dimers are very poor substrates to T4 polynucleotide kinase, and T = T was therefore treated with UVC to convert it to the easily labeled parental dinucleotide TpT, as previously described (11). The purified 40 μL sample was transferred into a 96-well plate and were exposed to 10 kJ/m^2 of UV radiation from a monochromatic 254 nm light UVC lamp (Stratalinker, La Jolla, CA). The UV-treated sample was transferred to a 500 μL Eppendorf tube, evaporated to dryness and TpT was ^{32}P-labeled on the 5′-side. The labeling mixture contained 0.2 μL of a buffer [20 mmol/L 2-(*N*-cyclohexylamino)ethanesulfonic acid, 10 mmol/L $MgCl_2$, 10 mmol/L dithiotreitol and 1 mmol/L spermidine (pH 9.6)], 1.4 μL of [γ-^{32}P]ATP (specific activity >6,000 Ci/mmol) and 3 units (0.1 μL) of T4 polynucleotide kinase (ATP and kinase were obtained from Amersham, Uppsala, Sweden). The sample was incubated at 37°C for 30 minutes, diluted with 5 μL water, and analyzed on the HPLC system equipped with an on-line radioisotope detector.

Duplicate samples of 10 fmol of the T = T standard were analyzed as an external standard with each set of human samples. The standard was treated in the same way as the samples, i.e., purified by HPLC, photoreverted, labeled, and analyzed by the ^{32}P-HPLC system. The amount of radioactivity in the ^{32}pTpT peak from the T = T standard was used for the calculation of T = T levels in the urine samples. All samples in the kinetic study were analyzed once; for the dose study, urine samples from days 1 to 5 were pooled and analyzed in duplicates. In order to correct for variations in urine concentration, T = T levels were adjusted for creatinine level, determined by Jaffe's method (12). Total urine volume was not measured but was assessed from 24-hour creatinine excretion, assuming 160 μmol creatinine per kg body weight per day for women and 200 μmol for men (10). For comparison between individuals, data were also adjusted for delivered UV dose and body surface area.

Purification of urine samples was done on a Beckman Instruments HPLC system (model 126 pump) operated with System Gold and coupled to a model 168 diode-array detector (Beckman Instruments, San Ramon, CA). The column used was a 5 μm, 4.6 × 250 mm reversed phase C18(2) Luna (Phenomenex, Torrace, CA). A precolumn filter was positioned before the column. The column was eluted isocratically with 50 mmol/L ammonium formate (pH 4.6) containing 5% methanol. The flow rate was 0.7 mL/min. ^{32}pTpT was analyzed on an HPLC system coupled to a model 171 radioisotope detector (all from Beckman Instruments). The C18 column used was a 4 μm, 2.0 × 250 mm Synergi Hydro-RP (Phenomenex), equipped with a precolumn filter. It was eluted with a linear gradient starting from 100% of 0.5 mol/L ammonium formate, 20 mmol/L orthophosphoric acid (pH 4.6), and going up to 20% methanol in 30 minutes. The flow rate was 0.2 mL/min and it was maintained using a split-flow device.

Results

HPLC analysis of ^{32}P-postlabeled TpT resulted in a radioactive peak of ^{32}pTpT with a retention time of 30 minutes (data not shown). Analysis of 10 fmol of the standard T = T after photoreversion to TpT and postlabeling gave rise to a radioactive peak with the same retention time (Fig. 1A). The recovery for standard T = T in the whole procedure (HPLC purification, photoreversion, postlabeling, and HPLC analysis) was >50%. The limit of detection was 0.5 fmol T = T in 10 μL of urine. If the collected HPLC fraction containing T = T was postlabeled directly, without irradiation with UVC, no radioactive peak was found at the retention time of pTpT (Fig. 1B). With the exception of a few individuals, urine samples contained nondetectable or very small amounts of T = T before tanning (Fig. 1C). Human urine collected 1 day after a tanning session always contained a radioactive peak at the retention time of pTpT, which was 30 minutes (Fig. 1D).

Urinary T = T levels increased gradually from the first day after irradiation to the highest levels on day 3, except for a few individuals, whose peak excretion of T = T was on day 2 or 4 (mean values shown in Fig. 2). T = T levels decreased gradually during the following days. The mean values for T = T excretion for days 0, 1, 2, 4, and 5 were all statistically significant from that at day 3 (the peak value). For most individuals, the increase from levels prior to UV exposure was >30-fold (compared with the limit of detection).

The average total amount of T = T excreted during days 1 to 5 was 3.0 nmol, resulting from an average erythema-weighted UV dose of 312 J/m^2. If normalized for dose and body surface area, the individual levels varied from 2.0 to 9.3 pmol per J/m^2 per m^2 body surface area. There was no effect of skin type or age on the total amount of T = T excreted (data not shown).

The cumulative T = T levels for the volunteers studied up to 11 days of solarium use are presented in Fig. 3. The increase in T = T levels was steep up to day 5, after which a plateau was reached. From the initial parts of the curves of accumulation (up to 5 days), a 50% excretion time was calculated (Table 1). For different individuals, the 50% excretion times varied between 55 and 76 hours.

Table 2 shows the individual data as well as mean values of total amounts T = T excreted during days 1 to 5, following the different exposure schemes. In the first session (A) the volunteers were exposed for 20 to 30 minutes, depending upon skin type, and the data were normalized to 30 minutes. Session B (half the exposure time of session A) gave, on average, 47% of A. Covering the upper half of the solarium, but using the same time as in A (session C) resulted in 44% of the T = T amount of session A. Repeating sessions A or B gave, on average, 105% and 46% of A, respectively.

Discussion

We have explored the use of urinary excreted T = T as a measure of the total body dose of UV. To deliver standardized doses, a commercial solarium for artificial tanning was used. The erythema-weighted UV dose for a 30-minute tanning session was 383 J/m^2 and the amount of UVB emission was 1%. These are typical data for solaria in commercial tanning

saloons in Sweden (13). For analysis of T = T, a ^{32}P-postlabeling assay with HPLC separation was used. With this method as little as 0.5 fmol of T = T could be quantified in just 10 μL of urine.

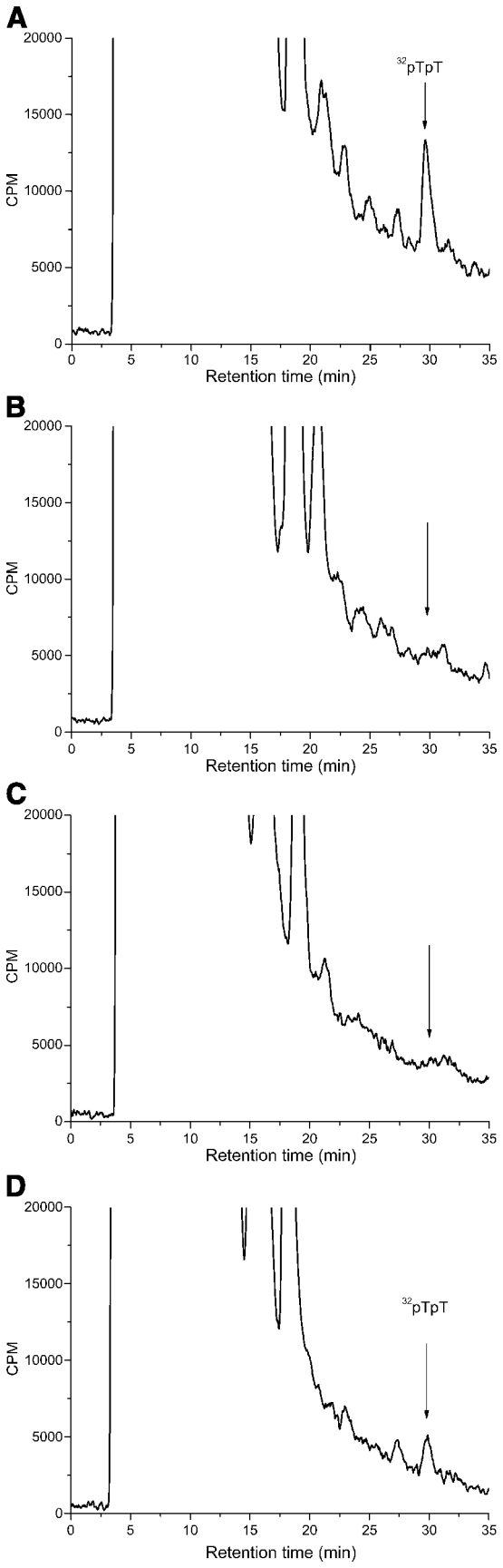

Figure 1. ^{32}P-HPLC chromatogram of thymine dimer (T = T). **A,** 10 fmol of T = T standard after photoreversion to parental dinucleotide followed by ^{32}P-postlabeling (^{32}pTpT). **B,** 10 fmol of T = T postlabeled directly without photoreversion. **C,** urine sample collected before solarium tanning. **D.** Urine sample collected 1 day after tanning (peak contains 4.3 fmol of ^{32}pTpT).

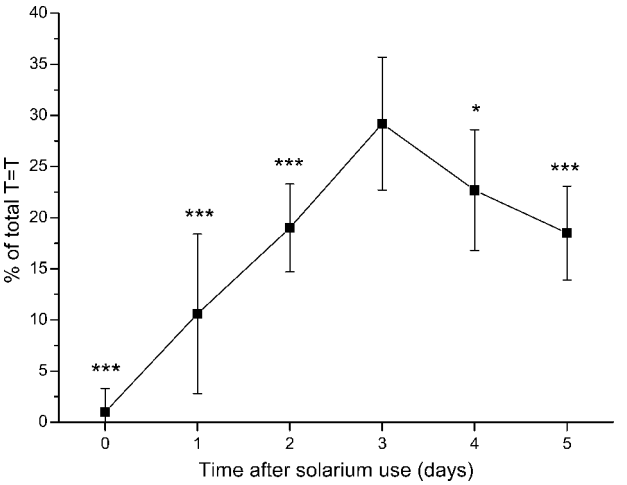

Figure 2. Kinetic of urinary thymine dimer (T = T) excretion after UV irradiation as mean values (± SD) for 10 persons during 20 to 30 minutes of solarium use. Time 0 represents the T = T level in samples taken just before the tanning session. The data for each day are expressed as a percentage of the total amount of T = T and compared statistically (*t* test) with day 3 (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Levels of T = T in urine samples collected before the tanning sessions were nondetectable or low in most individuals. The volunteers were asked not to stay in the sun and not use a solarium 1 week prior to the collection of the first urine sample. Nevertheless, some persons had clearly measurable levels of T = T (up to 6.1 fmol per 10 μL of urine). These observations suggested that these individuals were exposed to some sunlight during the week before tanning in the solarium, or that the background originated from an earlier sunbathing or solarium use. After completion of the present investigation, we studied background levels of urinary T = T in persons who worked full time and did not sunbathe or used a solarium. We noticed that during late spring, summer, and early autumn, these individuals occasionally had measurable T = T levels. This was not the case during late autumn or winter (data not shown). With this knowledge in hand, it would have been preferred to conduct the present study during the winter season. On the other hand, these data show the high sensitivity of the method used even in being able to pick up unintentional UV exposure.

T = T could be detected in all urine samples collected 1 day after tanning in the solarium and the levels increased steadily up to 3 days, after which they decreased (Fig. 2). The excretion for some individuals peaked at 2 or 4 days instead, possibly reflecting individual differences in repair rates. It has previously been shown that there are large differences between individuals in rates of repair of T = T from skin DNA *in situ* (14). The increase in T = T levels up to 3 days was dramatic (>30-fold for most individuals), which shows both that a single session in a commercial solarium is enough to produce high levels of T = T and that the nucleotide excision repair system is efficient in removing this lesion.

In a UV exposure study, similar to ours, urinary excretion of T = T following a single total body UV dose of 15 J/cm^2 was

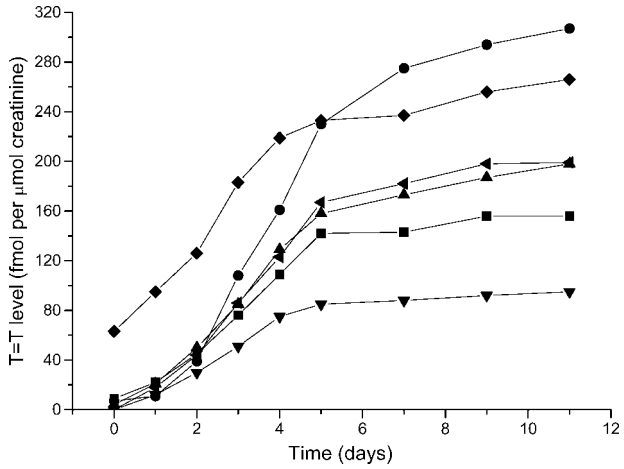

Figure 3. Cumulative thymine dimer (T = T) levels after UV irradiation for the six volunteers who were followed up to 11 days. Time 0 represents the T = T level in samples taken just before the tanning session.

analyzed using an immunologic method (15, 16). The results showed that T = T peaked at day 3 (15), which is consistent with our data. However, the increase in T = T levels from the prestudy levels was much less in the reported study compared with ours. The spectra of the lamps used in the two studies were very similar and the erythema-weighted dose of 383 J/m^2 used by us corresponds to an unweighted dose of 17 J/cm^2, i.e., very close to the 15 J/cm^2 used by Cooke et al. (15). The reasons for the discrepancies between the two studies are therefore unknown, but they could be due to differences in the assays used for T = T analysis.

The mean amount of the excreted T = T over days 1 to 5 for the 10 persons was 3.0 nmol, following an average UV dose of 312 J/m^2 (Table 1). If the excreted amount was standardized for dose and body surface area, it ranged for different individuals from 2.0 to 9.3 pmol, i.e., ~5-fold. This normalization did not change the ranges or the relative SDs of the data, demonstrating that other factors are more important for determining interindividual differences in urinary excretion of T = T. There was no correlation between skin type or age and total amount of T = T. This was maybe not unexpected because only skin types II and III were represented, and the population was small in size and similar in age, making it difficult to detect possible differences. In studies of T = T levels in human skin *in situ*, similar or even larger interindividual differences were noted (14, 17). The doses used in the latter studies were erythema-weighted and the observed interindividual variations could therefore not be explained by differences in skin types.

The kinetics of T = T excretion revealed differences between individuals regarding rates of T = T elimination (Fig. 3).Out of the six persons studied for up to 11 days, two subjects showed no or very little T = T after 5 days, whereas for the other four persons, small amounts of T = T were excreted each day up to day 11. Using the initial rate of excretion (days 1-5) a 50% excretion time was calculated (Table 1). The mean value was 68 hours and the difference between individuals was not large (55-76 hours). Differences in the rates of excretion between individuals depend on the elimination rates of T = T from skin DNA. We have previously found that, on average, 43% of T = T in the sequence TT = T was repaired from human skin DNA *in situ* in 48 hours, but with large differences between individuals (14). This elimination rate from skin DNA fitted with the urinary excretion data, with the exception that for the urinary excretion rates, the interindividual differences were much smaller than those in repair rates in skin (Table 1). The reason for this is unknown, but it is possible that the steps following repair, i.e., degradation of the excised oligomer and the excretion of the final product, T = T, would be rate-limiting.

Different conditions for solarium use were chosen to study how well urinary T = T levels would reflect the delivered UV dose (Table 2). The reference tanning lasted 20 to 30 minutes and the comparison included 50% of the time as well as 50% of the dose (half of lamps covered). Using half the time or half the number of lamps reduced, on average, the amount of T = T excreted during days 1 to 5 to 47% and 44%, respectively. Repeating the reference session or the one with half the time gave 105% and 46%, respectively. For certain individuals, the reduction in T = T levels for some sessions differed from the anticipated 50%. The reason for this could be the contribution of unintentional sun exposure prior to the test period (as discussed above). These data show that the amount of T = T excreted in the urine reproducibly correlates with the delivered UV dose. The very good correlation was somewhat unexpected because only single morning urine samples instead of total daily urine were collected. The latter would have been preferred but impracticable in a study consisting of 35 sessions of solarium use and 6 days of urine collection for each session. The T = T levels were corrected for creatinine excretion, which is the standard method of urinary analysis in clinical chemistry. However, correcting or not correcting for creatinine had very little effect on T = T levels because the creatinine levels did not differ much between the subjects and differed even less from day to day for a single individual (data not shown). It would have been desirable to compare the urine data with corresponding data from skin biopsies, and because we have the assays to do this (9), such a study is in our future plans.

In the present study, a quantitative and very sensitive assay was used for analysis of T = T in human urine. The obtained data show that the amount of excreted T = T was correlated

Table 1. Amounts of urinary thymine dimer (T = T) after UV irradiation in a solarium and the rates for its excretion

Volunteer	Sex	Dose (J/m^2)	Amount of T = T*, (nmol)	Amount of T = T (pmol per m^2 body surface area per J/m^2)	50% excretion time† (hours)
1	F	383	3.6	5.8	69
2	F	256	3.3	9.0	62
3	F	124	1.9	9.3	70
4	F	256	3.4	7.4	65
5	F	383	2.4	3.6	70
6	F	383	2.9	4.6	55
7	M	383	5.5	7.2	75
8	M	192	2.9	6.6	71
9	F	383	2.0	3.5	64
10	M	383	1.6	2.0	76

*Total amount of T = T for 5 days assuming an average excretion of 160 μmol creatinine per kg body weight per day for women and 200 μmol for men (10).
†The 50% excretion time was calculated from the excretion rate during the first 5 days following UV irradiation.

Table 2. Relative amounts of urinary thymine dimer excreted for 5 days following different UV sessions in a solarium

Volunteer	Percentage of session A				
	30-minute exposure (A)	50% of the time (B)	50% of the body (C)	Repeated A	Repeated B
1	100	26	32		29
2	100	40	29		58
3	100			97	
4	100	46	53		46
5	100	57		103	
6	100	50	69	122	
7	100	53	33		51
9	100	58	61	97	
10	100		31		
Average ± SD	100	47 ± 11	44 ± 17	105 ± 12	46 ± 12

NOTE: The data for session A were taken from the fourth column of Table 1 (volunteer 8 participated only in session A and was therefore not included). For session B, the exposure time was 50% of that in session A. For session C, the upper part of the solarium was covered resulting in 50% of the body being exposed. Sessions A and B were repeated once.

with the delivered UV dose. The present method estimates total body DNA damage and the rate of its repair. Moreover, being a noninvasive technique, the assay could also be used when studying children.

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