

Short Communication

Repair of UV Dimers in Skin DNA of Patients with Basal Cell Carcinoma

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Abstract

Epidemiologic studies suggest that exposure to sunlight is the primary etiologic agent for basal cell carcinoma. Formation of UV-induced DNA damage is believed to be a crucial event in the process leading to skin cancer. In this study, repair of photoproducts in DNA was followed in the skin of patients with basal cell carcinoma and control subjects. The subjects were exposed to 800 J/m² Commission Internationale de l'Éclairage of solar-simulating radiation on buttock skin. Biopsies were taken at 0 hour, 24 hours, and 3 weeks after the exposure. Two cyclobutane pyrimidine dimers, TT=C and TT=T, were measured using a sensitive ³²P-postlabeling assay. Initial levels of both TT=C and TT=T differed between individuals in both groups. The levels of TT=T in patients with basal cell carcinoma and controls were similar (9.9 ± 4.0 and 9.2 ± 2.9 products per 10⁶ normal nucleotides), whereas the level of TT=C

was significantly lower in controls than in patients with basal cell carcinoma (6.2 ± 3.1 versus 10.9 ± 4.5 products per 10⁶ normal nucleotides). The fractions of TT=T remaining after 24 hours and 3 weeks were significantly higher in patients with basal cell carcinoma (72% and 11%) compared with controls (48% and 5%). A slower removal in patients with basal cell carcinoma than in controls was indicated also for TT=C (52% versus 42% remaining at 24 hours); however, the difference between groups was not significant. When including data from our previously reported small-scale study, the fraction of dimers remaining at 24 hours was significantly higher in patients with basal cell carcinoma for both TT=C and TT=T. The data suggest that patients with basal cell carcinoma have a reduced capacity to repair UV-induced DNA lesions. (Cancer Epidemiol Biomarkers Prev 2008;17(9):2388–92)

Introduction

Basal cell carcinoma is the most common human cancer, and its incidence is increasing worldwide (1, 2). The predominant UV-induced lesions in DNA, which is the major epidermal chromophore, are the cyclobutane pyrimidine dimers (3). Photoproducts in DNA can alter the biological function of DNA and are believed to be a major contributor of the tumorigenic events that follow UV exposure (4–6). We have previously shown that high levels of cyclobutane pyrimidine dimers are formed in human skin *in situ* just after a brief exposure to solar-simulated UV radiation (7, 8). It is critical for the cell that these lesions are removed from DNA, an activity that is primarily carried out by the nucleotide (nt) excision repair pathway. The importance of this system is illustrated by the extreme UV sensitivity of patients with xeroderma pigmentosum, who lack a functional nt excision repair and therefore suffer a very high risk for developing skin cancer. In addition, a slower repair of UV damage for basal cell carcinoma cases than for

controls has been observed in several studies where DNA repair in lymphocytes or skin fibroblasts has been measured *in vitro* (9–11) and in one study in skin *in vivo* (12). Therefore, a reduced capacity to repair photolesions might be a risk factor for the development of this type of skin cancer.

In this study, we applied a sensitive ³²P-postlabeling method to detect two sequence-specific cyclobutane pyrimidine dimers in irradiated human skin *in situ* (7, 13). In an earlier study where cyclobutane pyrimidine dimers were measured 24 and 48 h after exposure, we found significantly higher levels of TT=C at 24 hours in patients with basal cell carcinoma compared with matched controls and an indication of a slower repair for cases than for controls (14). The aim of the present study was to further investigate the formation of cyclobutane pyrimidine dimers in patients with basal cell carcinoma and to study the repair of the lesions during an extended period of time.

Materials and Methods

A Corona Mini 220 (Essha Elangetur AB) UV source equipped with two Philips TL12 broadband UVB tubes was used. The spectral distribution of the lamp resembled the spectrum of noon summer sunlight in Helsinki (15). This study was conducted according to Declaration

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of Helsinki principles and approved by the Medical Ethics Committee of Päijät-Häme Central Hospital, Finland. Ten patients with basal cell carcinoma (mean age, 64.3 ± 12.9 years; range, 41-84 years) were recruited from subjects coming to the dermatologic clinic for a check up of their past or present skin cancer condition. They were interviewed to exclude familiar cases or syndromes connected with multiple basal cell carcinoma. Among the patients, 5 only had 1 basal cell carcinoma, 2 had 2 basal cell carcinomas, 1 had 3 basal cell carcinomas, 1 had 4 basal cell carcinomas (all on the nose, nodular), and 1 had 10 tiny basal cell carcinomas in the face (not any in family). All basal cell carcinomas were located at sunlight-exposed areas, face, neck, and some on the thoracic region, and were mainly of small nodular type. Ten individuals (mean age, 49.6 ± 7.7 years; range, 37-64 years) not diagnosed with basal cell carcinoma or any other type of skin cancer were recruited mainly from hospital staff members. There were two men and eight women in each of the two groups. The subjects gave their informed consent to participate in the study and were phototested for skin type. The subjects (one of skin type I, six of type II, two of type III, and one of type IV for the basal cell carcinoma group, and four of skin types I to II, three of type II, and three of type III for the control group) were exposed to an erythemally weighted UV dose of $800 \text{ J/m}^2 \text{ CIE (14)}$ on the buttock skin. Skin biopsies were taken within 15 minutes (0 hour), at 24 hours, and after 3 weeks. Dermis was separated from epidermis, DNA isolated from the latter, and cyclobutane pyrimidine dimers were analyzed by the ^{32}P -postlabeling assay as previously described (7) but with some modifications. Three micrograms of DNA (dissolved in 10 mmol/L Tris-HCl, 4 mmol/L MgCl_2 , pH 7.5) was digested with 7.5 mU of snake venom phosphodiesterase I (Sigma) during 4 hours at 37°C . Fifty mU of prostatic acid phosphatase (Sigma) was added, and incubation continued overnight. The next morning, 2.5 μg of proteinase K (Roche) was added, and the samples were incubated for 1 hour at 37°C . Proteins were precipitated by adding 100 μL of cold ethanol and centrifugation for 15 minutes at $17,000 \times g$. The supernatant was evaporated, and the samples were labeled by adding 2 μL of a labeling cocktail containing 0.2 μL buffer (0.5 mol/L Tris-HCl, 100 mmol/L MgCl_2 , 100 mmol/L 2-mercaptoethanol, pH 7.6) and 0.2 μL (6 U) T4 polynucleotide kinase (USB Corporation), 0.2 μL H_2O , and 1.6 μL [$\gamma\text{-}^{32}\text{P}$]ATP (3,000 Ci/mmol, GE Healthcare). The labeled samples were then analyzed by high-performance liquid chromatography equipped with an online radioisotope detector (Beckman Coulter). The reverse-phase column used was a $2 \times 250 \text{ mmol/L}$ Luna HC18(2) (Phenomenex) that was eluted at a flow rate of 0.2 mL/min with a buffer-methanol gradient starting with 100% 0.5 mol/L ammonium formate and 20 mmol/L phosphoric acid (pH 4.6). After 5 minutes, the methanol concentration was increased linearly to 8% during 5 minutes, followed by 8% methanol for 7 minutes, and finally, a linear gradient up to 78% methanol during 23 minutes. In this assay, each cyclobutane pyrimidine dimer is obtained as a trimer with a nonmodified nt on the 5' side of the dimer. Cyclobutane pyrimidine dimer levels, expressed as moles per 10^6 moles of normal nts, were calculated from the radioac-

tivity in the peaks of two major cyclobutane pyrimidine dimers TT=C and TT=T. Wilcoxon two-sample test was used for the statistical analysis.

Results and Discussion

Representative high-performance liquid chromatography chromatograms of the two cyclobutane pyrimidine dimers TT=C and TT=T in buttock skin samples from one individual at different time points after UV exposure are shown in Fig. 1. The upper chromatogram shows cyclobutane pyrimidine dimers present in the buttock skin directly after UV exposure; large peaks were detected for both TT=C and TT=T. After 24 hours, a significant fraction of TT=C and TT=T had been repaired as shown by the diminishing peaks (Fig. 1, middle chromatogram). Finally, 3 weeks after UV exposure, some TT=T still remained, but TT=C was not detected (Fig. 1, lower chromatogram).

The initial levels of TT=C and TT=T differed between individuals for both groups, up to approximately four times for TT=C and approximately two times for TT=T. Similar differences were seen also at the two later time points. Large interindividual differences have been observed in all human studies carried out in our laboratory (8). The mean levels of cyclobutane pyrimidine dimers for the two groups are shown in Table 1. The initial level of T-T = C for the cancer cases ($10.9/10^6 \text{ nt}$) was significantly higher than for the control group ($6.2/10^6 \text{ nt}$; $P = 0.023$). At 24 hours, the difference was still significant ($5.3/10^6 \text{ nt}$ for the basal cell carcinoma group and $2.4/10^6 \text{ nt}$ for the controls; $P = 0.015$). For TT=T, there were no significant differences between cases and controls at any time point. In our previous

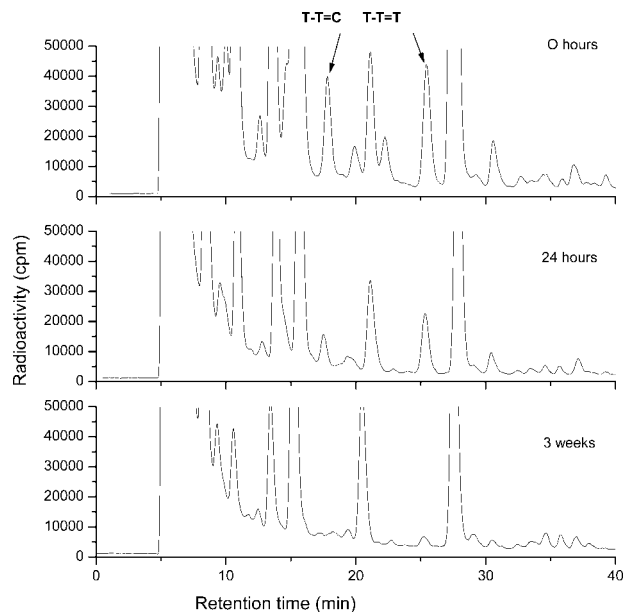


Figure 1. ^{32}P -High-performance liquid chromatography profiles of cyclobutane pyrimidine dimers in DNA from skin biopsies of a patient with basal cell carcinoma directly after UV exposure and 24 h and 3 wk thereafter.

Table 1. Photoproduct levels (per 10⁶ nts) and the percentage of photoproducts remaining at different time points after UV exposure in patients with basal cell carcinoma and controls (mean ± SD and the percentage of initial levels)

Time after exposure	Study*	TT=C absolute level (% remaining)		P value for % remaining (when excluding one person †)	TT=T absolute level (% remaining)		P value for % remaining (when excluding one person ‡)
		Controls	BCC cases		Controls	BCC cases	
0 h	This study	6.2 ± 3.1 (100)	10.9 ± 4.5 (100)		9.9 ± 4.1 (100)	9.2 ± 2.9 (100)	
	Ref. (14)	15.6 ± 12.1 (100)	12.8 ± 6.9 (100)		10.9 ± 6.2 (100)	9.5 ± 3.5 (100)	
	Pooled data	10.9 ± 8.8 (100)	11.8 ± 5.8 (100)		10.4 ± 5.2 (100)	9.3 ± 3.2 (100)	
24 h	This study	2.4 ± 1.5 (42)	5.3 ± 1.9 (52)	0.12 (0.25)	4.7 ± 2.0 (48)	6.4 ± 1.9 (72)	0.0052 (0.0090)
	Ref. (14)	3.6 ± 2.6 (29)	5.4 ± 3.5 (39)	0.086	5.2 ± 2.8 (62)	6.5 ± 2.9 (70)	0.55
	Pooled data	3.0 ± 2.1 (36)	5.4 ± 2.7 (48)	0.046 (0.061)	5.0 ± 2.4 (55)	6.4 ± 2.4 (70)	0.025 (0.035)
3 wk	This study	—‡	—‡		0.57 ± 0.62 (5)	1.00 ± 0.57 (11)	0.049 (0.072)

Abbreviation: BCC, basal cell carcinoma.

*This study includes 10 BCC patients and 10 controls. Ref. (14) includes 9 BCC patients and 10 controls.

†P value when excluding one patient who had multiple basal cell carcinomas.

‡Below the detection limit of 0.1/10⁶ normal nts.

study of patients with basal cell carcinoma (14) and in the study by Alcalay et al. (12), the initial levels of cyclobutane pyrimidine dimers were similar in the two groups. Thus, it is unclear whether patients with basal cell carcinoma are more sensitive than healthy individuals to induction of UV lesions in DNA. However, one study has indicated increased sensitivity of basal cell carcinoma cases to *in vitro* UVB-induced chromatid breaks (16).

The data on rates of repair (Table 1) confirmed our earlier observation that TT=C is repaired faster than TT=T (14, 17-19). The same sequence specificity in repair of cyclobutane pyrimidine dimers has been observed for UV-exposed human skin explants (20). The authors suggested that the reason could be that different cyclobutane pyrimidine dimers might induce different structural effects on DNA or simply that the cyclobutane pyrimidine dimer sequence might influence the binding of nt excision repair proteins to the DNA and thus modify the efficiency of repair. More importantly, we observed that 24 hours after UV exposure, the patients with basal cell carcinoma had significantly more TT=T remaining than the control subjects (72% compared with 48%; $P = 0.0052$). In addition, for TT=C, there was a tendency toward a larger fraction remaining in the cases than in the controls (52% versus 42%; $P = 0.12$). At 3 weeks, approximately 11% of the initial amount of TT=T remained in the basal cell carcinoma group compared with 5% in the control group. This difference was statistically significant ($P = 0.049$). TT=C was not detected in any of the individuals at the last time point. In our earlier study of a group of patients with basal cell carcinoma, we found that basal cell carcinoma cases had a lower capacity to repair cyclobutane pyrimidine dimers compared with matched controls. The difference between cases and controls was not significant for TT=T but was significant for TT=C at 24 hours (the time points 24 and 48 hours were investigated; ref. 14). Furthermore, the percentage of cyclobutane pyrimidine dimers remaining at 24 hours was similar to what we found in the current study (for both cases and controls). When pooling the data from the two studies, the difference between cases and controls in percent TT=T remaining at

24 hours (70% versus 55%) was still significant ($P = 0.025$), and in addition, the difference in amounts of TT=C left in DNA at the same time point (48% versus 36%) became significant ($P = 0.046$). Figure 2 shows median values and variability of the remaining fractions of TT=C and TT=T, respectively, 24 hours after the exposure (pooled data). The plot shows the large interindividual variation in rates of cyclobutane pyrimidine dimer repair.

The criteria for selection of study subjects and the assay for cyclobutane pyrimidine dimer analysis were identical in the two investigations. The UV lamps were different but gave the same emission spectrum (15), and the CIE-weighted dose was two times higher in the current study (800 and 400 J/m², respectively). In

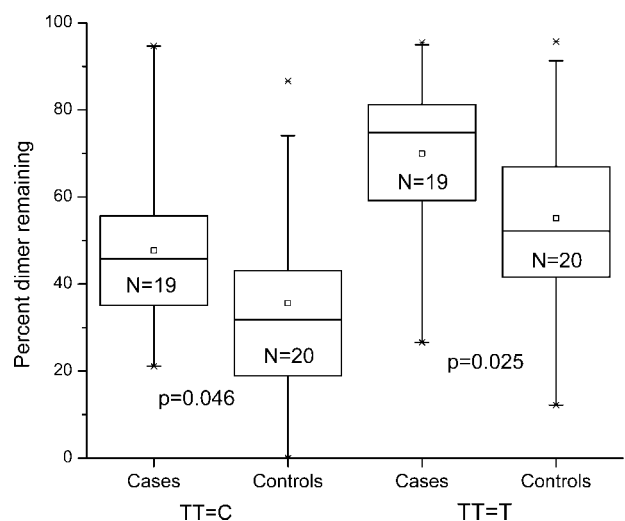


Figure 2. Median values and variability of the remaining fractions of TT=C and TT=T, respectively, in skin biopsies from patients with basal cell carcinoma ($n = 19$) and from control subjects ($n = 20$) 24 h after UV exposure. Data from the current study have been pooled with data from ref. 14.

both investigations, the distribution of skin types was similar, as well as the basal cell carcinoma type (mainly nodular), location (mainly facial), and the number of tumors (50% of cases with 1 basal cell carcinoma). The variation between duplicate samples analyzed was 47% in this study compared with 45% in the earlier investigation. In the present study, but not in the earlier one (14), the range of ages among the cases was wider than for the controls. We have previously investigated the effect of age on DNA repair, but no effect was found (18). Over the years, repair data have been collected from ~150 individuals with an age range from 20 to 84 years, and no correlation between age and DNA repair capacity has been found (data not shown). Therefore, age is not expected to explain the observed differences between the two groups. One of the cancer patients had multiple basal cell carcinoma in the face and developed additional ones after this study was completed, and the dermatologist involved in the case suspects that it could be Gorlin syndrome. It is interesting to note that this person was among the slowest in repair of both TT=C and TT=T, and if excluding the data for this individual, the *P* value for TT=C at 24 hours (pooled data) and for TT=T at 3 weeks (this study) is increased above 0.05 (Table 1). However, most literature data do not support a direct involvement of nt excision repair in this condition (21). Thus, it is unlikely that this person differs in this respect from the other basal cell carcinoma cases.

The results obtained in our two studies and in a study where cyclobutane pyrimidine dimers were analyzed, using a dimer-specific endonuclease, in skin biopsies 0 and 6 hours after UV irradiation (12) indicate, together with findings in studies where the host reactivating and other assays have been used (9-11), that patients with basal cell carcinoma have less efficient DNA repair compared with healthy individuals. The mechanism(s) behind this decreased DNA repair capacity is unknown. Possibly, the expression of genes involved in the DNA repair process could be modified by UV light specifically in individuals who would be prone to developing basal cell carcinoma. Several of the genes involved in nt excision repair have been shown to be polymorphic, although information of the function of these variants is scant. The observed differences in repair between patients with basal cell carcinoma and control subjects could be due to such variants in DNA repair genes. However, association studies carried out thus far have given conflicting results (22-28). To further strengthen links between DNA repair and risk for basal cell carcinoma, studies with more subjects would be needed to compensate for the relatively large interindividual variation. Substantially larger study groups than the one used here would also be needed to study possible effects of genetic polymorphisms on DNA repair capacity. Collaboration between DNA repair research groups could increase the power of these studies; harmonization of sampling schedules and analytic methods would allow assessment of the results by means of statistical tools.

Disclosure of Potential Conflicts of Interest

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