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Epidermal Urocanic Acid Concentration and Photoisomerization Reactivity in Patients with Cutaneous Malignant Melanoma or Basal Cell Carcinoma

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The relationship of epidermal urocanic acid concentration and photoisomerization reactivity to human skin cancer was studied. Twelve cutaneous malignant melanoma patients, 10 basal cell carcinoma patients and 22 healthy matched controls were enrolled in the study. A solar simulating ultraviolet irradiator was used for phototesting the minimal erythema dose. Using the Finn Chamber® technique, urocanic acid was sampled from the healthy skin of the upper back, prior to and after exposure to suberythemal UV doses. The mean values of total and *trans*-urocanic acid were higher in basal cell carcinoma patients than in controls, but this difference was not statistically significant. No corresponding phenomenon was evident in the case of cutaneous malignant melanoma patients and their controls. Photoisomerization induced by irradiation with 1 mJ/cm^2 CIE (Commission Internationale de l'Éclairage) was statistically significantly lower in cutaneous malignant melanoma patients than in controls ($p = 0.04$). A similar trend was seen in basal cell carcinoma patients vs. their controls, but the difference was not significant. *Key words: skin neoplasms; UV radiation; photoreceptors.*

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Ultraviolet radiation is regarded as the most important aetiological factor in skin cancers, including cutaneous malignant melanoma (CMM). This conclusion is derived both from animal studies, from epidemiological data on the occurrence of skin cancer and from the detection of a global latitudinal gradient for CMM (1–5). UV exposure of DNA generates a variety of photoproducts, of which the pyrimidine dimers are the most frequent (6). In addition, UV radiation downregulates immunological responses, which may lead to inefficient detection and elimination of cancer cells. The *cis*-isomer of epidermal urocanic acid (UCA) plays an important role in UV radiation-induced immunosuppression (7).

Subjects presenting with photosensitive skin phototypes have been shown to be more prone to UV-induced skin cancers than subjects with phototolerant skin phototypes (3). However, a recent study of healthy volunteers failed to show any obvious differences in epidermal total UCA concentrations between sun-resistant and sun-sensitive skin phototypes (8). Interestingly, in an early study, scrapings from biopsies of malignant skin lesions were reported to contain no or minimal UCA and to be devoid of histidase enzyme activity, in sharp contrast to benign lesions (9). Unfortunately, the UCA levels of the uninvolved skin were not assessed in that study, and the possible influence of epidermal UCA levels on carcinogenesis susceptibility has remained unstudied. In the present study, we analysed the epidermal UCA isomer levels and UCA photoisomerization reactivity in patients suffering from CMM or basal cell carcinoma (BCC). Samples were also taken from matched controls.

MATERIAL AND METHODS

Patients and volunteers

The study protocol was approved by the Ethics committee of the Päijät-Häme Central Hospital and the patients and the volunteers gave their informed consent to participation in the study. Altogether 22 patients with skin cancer and an equal number of matched control subjects were included. Twelve patients (3 females and 9 males) had recently been treated for CMM and 10 patients (7 females and 3 males) had been treated for BCC. At the time of examination the mean age of the CMM patients was 51 years (range 31–75 years) and the mean age of the BCC patients was 66 years (range 50–76 years). The study was implemented in the winter, i.e. from the beginning of November 1996 to the end of March 1997, so that the time interval from the last sunlight exposure was at least 2 months, and the subjects had not exposed themselves to artificial UV radiation in that time period.

In one patient 4 separate melanomas had been detected and another CMM patient had fatal metastases with jaundice. Four patients had had more than 1 BCC. All BCC patients had had at least 1 lesion on the skin of the face. Eight patients had presented with nodular BCCs and 2 patients with superficial types of BCC.

The control subjects were recruited from staff members, patients with an unrelated skin disease, or relatives of the cancer patients or the staff. None of the control subjects had been treated for any skin malignancy. In addition, their skin was examined at the time of inclusion. The control subjects were matched with the skin cancer patients for the age (± 5 years), gender and anamnestic phenotype as regards the photosensitivity (10). Since the Fitzpatrick's anamnestic skin phototyping has proved to be unreliable (11–15), we combined the anamnestic Caucasian skin phototype classes I and II to a group of photosensitive subjects and the anamnestic Caucasian skin phototype classes III and IV to a group of phototolerant subjects. Thus defined, 7 of the 12 CMM patients and 8 of the 10 BCC patients and the corresponding numbers of controls were classified as photosensitive, while the remaining individuals were phototolerant. The mean age of the matched CMM controls was 50 years (range 35–71 years), and that of the BCC controls 66 years (range 47–76 years).

UV source and calibration

A solar simulating light source Philips HP 411/A (15) was used for phototesting as well as for inducing photoisomerization in the UCA sampling areas. The irradiance of the lamp was measured as 250–400 nm prior to the study at 30 cm using an Optronic 742 spectroradiometer with Teflon diffuser as input optics. The spectroradiometer is a temperature- and wavelength-controlled instrument, which was calibrated against a 1000 W halogen standard lamp trace-

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able to the National Institute of Standards and Technology (USA). The uncertainty of the spectroradiometric measurements was estimated to be about ± 10%. According to the measurements the erythemally weighted UV dose of 0.5 mJ/cm^2 CIE (Commission Internationale de l'Éclairage) was obtained at the distance of 30 cm in 11 s and 1.0 mJ/cm^2 CIE in 23 s (16).

Phototesting

In order to define the minimal erythema dose (MED) and the minimal perceptible erythema dose (MPED), all volunteers were phototested using the solar simulator with UV test doses of 10, 14, 20, 28, 40 and 57 mJ/cm^2 CIE (15). The phototests were read at 24 ± 2 h after the UV exposure. The MPED was defined as the minimal erythema visible to the eye, and the MED as the UV dose causing a faint but definitely sharp-bordered erythema in the test area.

UCA sampling

Samples for UCA measurements were obtained from the upper back of the test subjects using a non-invasive Finn Chamber® method described earlier (17). The samples were taken in triplicate using a 0.5 ml volume of 0.1 M KOH per sample. Each disc was prior to sampling moistened with 17 µl KOH, which is the volume of the Finn Chambers®. The moistened discs were applied to the skin for 30 min and then removed and put in groups of 3 in the test tubes, which were kept in the dark at room temperature for 24 h. Thereafter the discs were removed and the liquid phase deep frozen and analysed within 6 months using HPLC (18). The mean of triplicate samples was used in calculations, except in 2 instances, where only 2 parallel samples were available. UCA samples were obtained both from unexposed healthy skin, and from skin area, which prior to sampling was irradiated with 0.5 mJ/cm^2 or 1.0 mJ/cm^2 CIE doses of solar simulating UV radiation. During irradiation, the skin to remain unexposed was protected using an opaque plastic and cotton shield. UCA was sampled immediately thereafter.

Statistical analysis

For statistical evaluation between individuals the 2-sample rank sum test (Mann-Whitney) was used. The Wilcoxon 1-sample (signed rank sum) test was used to analyse the significance of UCA photoisomerization reactivity within groups (19).

RESULTS

The median MPED was 20 mJ/cm^{2}CIE in CMM patients and 17 mJ/cm^2 CIE in their control group, while the median MED values were 28 mJ/cm^2 CIE both in the CMM and the control group (Fig. 1). In both the BCC patients and their controls, the median MPED was 20 mJ/cm^2 CIE. The median MED was 20 mJ/cm^{2}CIE in BCC patients and 28 mJ/cm^{2}CIE in their matched controls (Fig. 1). No statistically significant difference was found between the patient group (CMM or BCC) and its control group.

In non-irradiated uninvolved skin, the mean values of total and *trans*-UCA were higher in BCC patients than in their matched controls (Table I), but the differences were not statistically significant; no corresponding trend was evident in the case of CMM patients and their controls. The individual values of total UCA in non-irradiated skin shown in Table II varied from a minimum of 2.6 nmol/cm^2 to a maximum of 62.1 nmol/cm^2. In individual cases the mean and median of the triplicate samples were consistent and in general the SD values were 20 – 30% of the mean. The patients with more than one BCC (subjects 4 and 7 – 9, Table II) and the patient with multiple treated CMMs (subject 11, Table II) or with metastases (patient 7, Table II) did not differ substantially from the other patients or control subjects as regards the UCA concentrations.

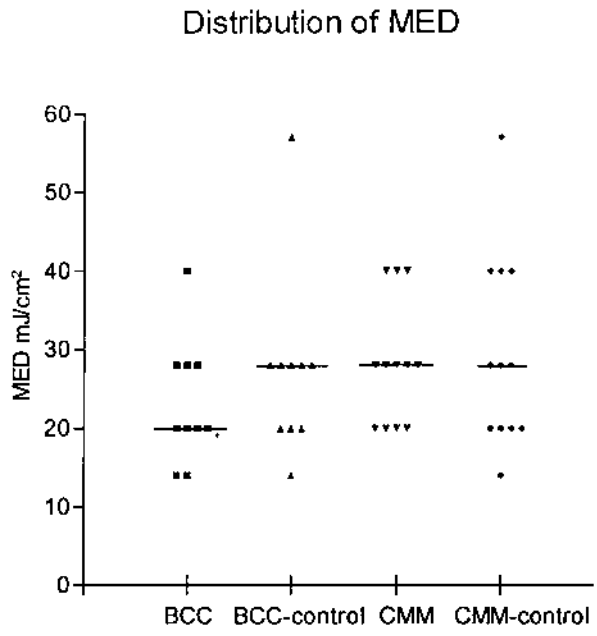

Fig. 1. Distribution of individual (symbols) and median (solid lines) MED values mJ/cm^{2}CIE in basal cell carcinoma (BCC) and cutaneous malignant melanoma (CMM) patients and their matched controls.

Even the small UV dose of 0.5 mJ/cm^2 CIE induced a statistically significant epidermal UCA isomerization in all 4 test subject groups (Table I). The isomerization rate induced by 1.0 mJ/cm^2 CIE was statistically significantly ($p = 0.04$) lower in CMM patients than in their matched controls (Fig. 2). A similar trend was seen in the case of BCC patients and their controls, but the difference was not statistically significant.

DISCUSSION

In contrast to an earlier publication, indicating loss of UCA synthesis in BCC samples (9), we found that epidermal UCA

Table I. *Concentrations (mean and SD) of total, trans- and cis-urocanic acid (UCA) nmol/cm^{2}in unirradiated and irradiated skin of the probands*

	UV-dose mJ/cm^2	*n*	Total UCA nmol/cm^2	*Trans*-UCA nmol/cm^2	*Cis*-UCA nmol/cm^2
CMM	–	12	11.9±5.3	11.4±5.2	0.5±0.2
CMM controls	–	12	13.6±6.6	13.0±6.2	0.6±0.4
BCC	–	10	23.9±16.3	22.9±15.7	1.0±0.8
BCC controls	–	10	13.7±8.6	13.1±8.3	0.7±0.5
CMM	0.5	12	13.8±4.9	12.9±4.7	0.9±0.3
CMM controls	0.5	12	15.6±6.2	14.5±5.9	1.1±0.5
BCC	0.5	10	27.1±17.0	25.6±16.5	1.5±0.8
BCC controls	0.5	10	16.3±10.1	15.2±9.9	1.1±0.6
CMM	1.0	12	16.1±6.1	14.9±5.7	1.2±0.5
CMM controls	1.0	12	17.3±7.3	15.6±6.9	1.7±0.8
BCC	1.0	10	29.5±16.9	27.5±16.3	2.0±0.8
BCC controls	1.0	10	18.7±8.5	17.2±8.3	1.5±0.7

CMM, cutaneous malignant melanoma; BCC, basal cell carcinoma.

Table II. *Gender, age, skin phototype and total urocanic acid (UCA) concentration (nmol/cm^2) in the unexposed skin of the probands*

Subject	Gender	CMM			CMM control		
		Age (years)	Skin phototype	Total UCA (nmol/cm^2)	Age (years)	Skin phototype	Total UCA (nmol/cm^2)
1	F	56	I – II	15.9	56	I – II	27.0
2	M	50	I – II	17.6	46	I – II	13.3
3	F	32	I – II	14.8	35	I – II	12.4
4	M	48	I – II	14.7	47	I – II	5.2
5	M	39	I – II	17.2	43	I – II	6.8
6	F	31	I – II	13.6	35	I – II	6.0
7	M	50	I – II	8.2	50	I – II	18.7
8	M	58	III – IV	9.3	53	III – IV	21.7
9	M	75	III – IV	8.2	71	III – IV	16.3
10	M	60	III – IV	2.6	60	III – IV	9.3
11	M	48	III – IV	3.7	44	III – IV	12.6
12	M	62	III – IV	16.9	63	III – IV	13.9

Subject	Gender	BCC			BCC control		
		Age (years)	Skin phototype	Total UCA (nmol/cm^2)	Age (years)	Skin phototype	Total UCA (nmol/cm^2)
1	F	71	I – II	32.6	74	I – II	14.0
2	M	53	I – II	7.6	56	I – II	7.5
3	F	68	I – II	62.1	72	I – II	2.9
4	M	67	I – II	12.8	69	I – II	12.7
5	F	76	I – II	37.4	74	I – II	9.1
6	F	50	I – II	14.2	47	I – II	18.6
7	F	75	I – II	22.2	76	I – II	30.6
8	M	72	I – II	16.4	70	I – II	9.3
9	F	67	III – IV	21.4	66	III – IV	24.6
10	F	58	III – IV	12.7	59	III – IV	8.0

CMM, cutaneous malignant melanoma; BCC, basal cell carcinoma.

levels of symptom-free skin of BCC patients were not decreased, but that they demonstrated a trend towards elevated values (Table I). The data, however, are not necessarily contradictory, since diminished UCA synthesis could be limited to the site of a cutaneous neoplastic process, while a preceding elevation of epidermal UCA synthesis could be connected to an epidermal predisposition for cancer development (20). The trend for elevated UCA levels in symptom-free epidermis of BCC patients, however, did not reach statistical significance, and no such trend was evident in CMM patients (Table I). A similar trend for higher total UCA values in the upper back of BCC patients compared with control subjects is evident in a recent paper (21). However, the limited number of patients (12 and 10 plus controls) in our study does not allow further conclusions. In addition, we have to take into account that some of the healthy volunteers may later on develop either BCC or CMM.

While epidermal UCA levels vary greatly between individuals (21 – 23, and data shown in Table II), no long-term follow-up data of epidermal UCA levels have been published. Earlier, an animal study showed that mice given a histidine-rich diet displayed significantly increased total UCA levels compared with mice fed a normal diet (24). The possible effect of diet on human epidermal UCA concentrations was not addressed in our study. In further studies the interindividual variation should be studied more thoroughly. If individuals do segregate into steady phenotypes of low vs. high UCA expressers, a long-term follow-up of large enough populations would be needed to define the role of UCA in the molecular epidemiology of skin cancer. The Finn-Chamber® method of UCA sampling is quite suitable for such studies, due to its simplicity and reliability (8, 21).

The choice of the low doses of 0.5 and 1.0 mJ/cm^2 was based on our earlier studies (8) and was aimed at producing recordable isomerizations, while avoiding running into photostationary isomerization dose range (25); it does not reflect any attitude to what dose ranges are operative in cutaneous photocarcinogenesis. In the present study, CMM patients isomerized statistically significantly less UCA than did their matched controls after a standard irradiation dose of 1 mJ/cm^2 (Fig. 2). We have no explanation for the mechanistic or biological relevance of this phenomenon. On the contrary, an increased isomerization propensity could have been anticipated, since *cis*-urocanic acid is an immune suppressive substance (7) and UV-induced immune suppression has been shown to be more readily induced in skin cancer patients than in their controls (26). We conclude that any crucial role of epidermal urocanic acid synthesis and UV-induced photoisomerization in the development of human skin cancer remains unproven.

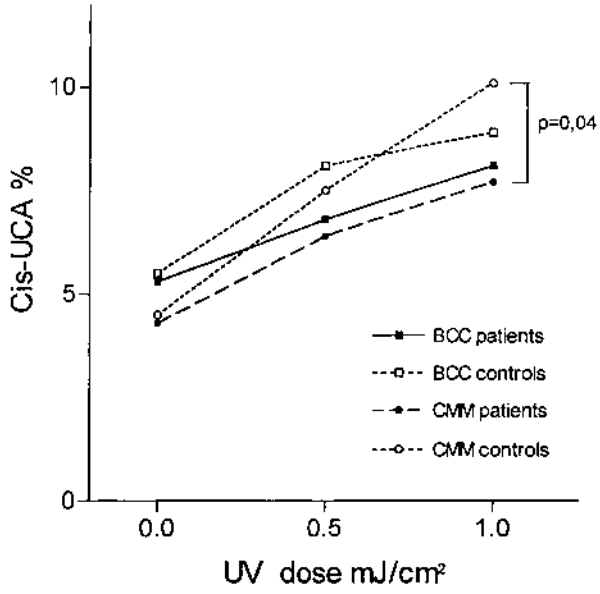

Fig. 2. The mean *trans*- to *cis*-urocanic acid (UCA) isomerization reactivity in basal cell carcinoma (BCC) and cutaneous malignant melanoma (CMM) patient and control groups induced by erythemally weighted UV doses of 0.5 and 1.0 mJ/cm^2 Commission Internationale de l'Éclairage. The mean values shown are derived from the *cis*-UCA% of each individual.

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