ylated indole derivatives in melanoma urine indicates formation in the melanocyte.

The urinary excretion of 5,6-DHI-2-C did not increase with increasing concentration of 5-S-cysteinyldopa. This finding is similar to that obtained with serum dopa concentrations which were shown to remain at a normal level also in patients with melanoma metastases—except for one patient with an extremely high serum 5-S-cysteinyldopa concentration (2).

Complex mixtures of indole metabolites have been used in the diagnosis of malignant melanoma. Due to the instability of 5,6-DHI-2-C this compound will probably not be suitable for routine analysis of melanoma urine, but investigations on dopa metabolism and pigment formation should in the future also include this new substance.

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The Effect of UVB Light on Serum Concentrations of 5-S-Cysteinyldopa

E. Tegner, G. Agrup and E. Rosengren

Departments of Dermatology and Pharmacology, University of Lund, Lund, Sweden

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Abstract. 5-S-Cysteinyldopa concentrations in serum were studied in patients with psoriasis treated with UVB light. An increase in 5-S-cysteinyldopa was found after three treatments. The highest values were noted after 5 weeks' treatment.

Key words: 5-S-Cysteinyldopa; UVB light; Melanocytes; Psoriasis

There is a current trend back to the use of UVB phototherapy for psoriasis (2, 3, 9, 10, 11). Comparative studies have been made concerning the efficacy of phototherapy and photochemotherapy (15). Exposure to sunlight leads to increased urinary excretion of the melanocytic metabolite 5-S-cysteinyldopa (13). In a series of investigations we studied the effect of various treatments with artificial light on 5-S-cysteinyldopa (1, 7, 14). We have monitored the urinary excretion and serum concentrations of 5-S-cysteinyldopa in patients with psoriasis treated with PUVA, and found a marked increase in 5-S-cysteinyldopa after 3 days' treatment, at which time no pigmentation had yet appeared. The highest concentrations in urine and serum were noted after 1 to 3 weeks' treatment (1, 7). Recently we have also studied 5-S-cysteinyldopa concentrations in serum in healthy individuals after exposure to UVA light alone, and found a marked increase after 3 days' irradiation and still higher individual concentrations after 7 to 10 days (14). The aim of the present study was to observe the chemical events in the melanocytes after exposure to artificial UVB light as reflected by changes in serum concentrations of 5-S-cysteinyldopa.

MATERIAL AND METHODS

12 otherwise healthy psoriasis patients were included in the study. All were outpatients. The extent of the skin lesions varied between 10% and 50%, i.e. only moderate psoriasis; patients with more generalized disease are given treatment other than UVB light alone. All 12 patients

Table I. *Skin type and pigment data in 12 patients with psoriasis*

The following criteria were used: skin type I = always burn, never tan; II = always burn, then slight tan; III = sometimes burn, always tan; IV = never burn, always tan

Patient	Age	Sex	Hair colour	Eye colour	Skin type
O. S.	39	M	Blond	Blue	II
P. P.	35	M	Brown	Brown	II
B. J.	17	F	Brown	Blue	II
I. P.	71	F	Brown	Blue	III
A. A.	29	M	Blond	Blue	III
O. H.	74	M	Brown	Brown	III
M. E.	31	F	Blond	Blue	III
Å. B.	52	M	Brown	Green	III
G. F.	49	F	Blond	Blue	III
I. B. L.	61	F	Brown	Blue	III
T. J.	15	M	Brown	Blue	IV
G. J.	35	F	Brown	Brown	IV

were of the opinion that exposure to sunlight improved their psoriasis. None were receiving any drugs. There had been no exposure to strong sunlight or other UV radiation during the 2 to 3 months preceding the investigation, which was made during November and December to avoid the influence of solar exposure (13).

The light source was a UVB cabin (Waldmann Radiation Unit UV 1000) with an emission spectrum between 280 and 380 nm and a peak emission of 313 nm. The intensity of the lamp in the UVB region was measured with a Waldmann-UV-Meter. The relative spectral sensitivity of the UV-meter has a spectrum of approx. 285–350 nm, with a maximum between 310 and 315 nm. At a distance of 20 cm from the tubes the intensity was estimated to 2.2 mW/cm^2. The patients received four treatments per week. The initial dose was 20–40 sec, depending on skin type (Table I). As a rule the exposure time was increased by 20–30 sec at each session. The exposure times were chosen to give slight erythema throughout the treatment period.

10-ml venous blood samples for 5-S-cysteinyldopa analysis were collected in glass tubes containing 10 mg sodium metabisulphite before the UVB treatment series started, and after 1, 3, 7, 10, 14 and 21 treatments. In 7 patients a further blood sample was collected after 28 treatments. The samples were centrifuged at 4500 rpm for 10 min within 1 h. Serum was precipitated with 1/10 volume 4 M perchloric acid, centrifuged at 15 000 rpm, and filtered. 5-S-Cysteinyldopa was adsorbed onto Al_2O_3 at pH 8.6, eluted with 1 ml 1 M perchloric acid, and then determined by high-performance liquid chromatography (HPLC) and electrochemical detection (4).

RESULTS

The psoriasis lesions improved in all patients, and in some they even cleared up after 21 to 28 sessions, i.e. 5–7 weeks' treatment. UVB was given with the aim of eliciting slight erythema or a slight burning sensation after each exposure, and slight erythema was therefore noted in all patients after almost each exposure. If the previous exposure had caused more than slight erythema, the same exposure time was used again. In none of the patients did treatment have to be stopped because of discomfort. All patients developed tanning, which was most pronounced in those of type IV (Table I).

The serum concentration of 5-S-cysteinyldopa before irradiation was 1.5–5.9 ng/ml serum (mean 2.6 ng/ml) (Table II), i.e. within the previously defined normal range (5, 6). After three treatments 5-S-cysteinyldopa values had increased in all patients (Table II and Fig. 1); by that time erythematous reactions had occurred in all, but no increase in pigmentation could be seen. After 10 treatments the mean serum concentration was almost 2½ times greater than the starting value, and after 21 treatments it was even higher. In 7 patients treatment was continued; the serum values after 28 treatments are shown in Table II. In 2 (B. J. and M. E.) the serum 5-S-cysteinyldopa had increased further, but in the other 5 the values were lower than after 21 treatments.

DISCUSSION

All patients showed an increase in serum concentrations of 5-S-cysteinyldopa after three treatments.

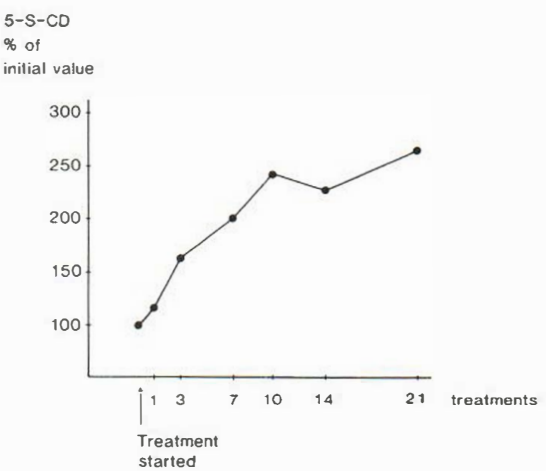

Fig. 1. Mean serum concentrations of 5-S-cysteinyldopa in 12 psoriatics during 5 weeks' UVB treatment (initial value = 100 %).

Table II. *Serum concentrations (ng/ml) of 5-S-cysteinyldopa during 5 to 7 weeks' treatment*

Patient	Before treatment		No. of treatments 1	3	7	10	14	21	28
O. S.		2.4	2.8	4.4	5.3	5.9	6.9	7.2	
P. P.		2.1	1.8	2.2	3.9	6.1	6.8	6.9	
B. J.		1.9	4.0	2.2	3.0	3.5	2.3	5.9	9.4
I. P.		2.7	2.5	6.2	3.1	6.3	7.6	7.7	6.8
A. A.		2.7	3.2	3.9	4.1	4.4	3.2	4.0	
O. H.		5.9	4.5	8.2	7.5	6.1	4.3	7.6	6.3
M. E.		2.7	3.4	3.7	6.6	6.4	5.8	4.8	7.1
Å. B.		2.4	2.7	3.7	6.1	6.7	4.4	3.9	
G. F.		1.5	1.6	4.8	6.6	8.5	6.7	7.3	
I. B. L.	3.0	2.6	2.9	3.1	4.7	5.8	6.0	6.1	3.7
T. J.	3.3	2.1	2.5	3.9	5.1	5.5	5.1	6.2	5.8
G. J.		2.7	3.0	3.7	2.6	5.2	5.8	8.6	5.2

The mean serum concentration was greatest after 21 treatments, but there were individual variations (Table II). PUVA treatment leads to a greater increase in serum 5-S-cysteinyldopa, and PUVA patients who developed pronounced erythema showed very high 5-S-cysteinyldopa values at the time of the erythematous reaction (1, 7). After exposure to UVA light alone the serum 5-S-cysteinyldopa concentrations increased to about the same level as in the present study, but this level was reached earlier, after only 4 days (14). In the UVA study, a great increase in serum value was noticed in the type I individuals, who developed marked erythema. Thus it has previously been observed that the most pronounced increases in 5-S-cysteinyldopa occur after UVA or PUVA leading to erythematous reactions. 5-S-Cysteinyldopa response is apparently more closely related to cell damage than to the degree of pigmentation resulting from the irradiation.

With UVB the erythema response was stronger, while the pigmentation was weaker than with UVA or PUVA. However, the 5-S-cysteinyldopa response was similar to that following UVA irradiation. Our treatment schedules with UVB and UVA were different, and direct comparison of the cysteinyldopa responses is not possible. Nevertheless, the present findings are compatible with our earlier hypothesis that elevated 5-S-cysteinyldopa levels are more closely related to cell damage than to pigment response (1, 7).

Repeated exposures to UVB is known to cause a marked increase in the number of active melanocytes and in the number of melanosomes (8, 12). The observed increase in 5-S-cysteinyldopa may be related to increased numbers of melanocytes and increased activity in the melanocyte.

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Methaemoglobin-catalysed Formation of Dopa and 6-OH-Dopa from Tyrosine

G. Agrup, C. Hansson, H. Rorsman, E. Rosengren and E. Tegner

Departments of Dermatology and Pharmacology, University of Lund, Lund, Sweden

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Abstract. An extract of bovine retina and choroid with tyrosine hydroxylating and dopa-oxidizing capacity also showed marked formation of 6-OH-dopa on incubation with tyrosine and ascorbic acid. The extract contained appreciable amounts of methaemoglobin as determined spectrophotometrically, and boiled extracts also showed catalytic activity. The effect of methaemoglobin on the oxidation of tyrosine and dopa was therefore investigated. Methaemoglobin catalysed the formation of dopa and 6-OH-dopa in the presence of tyrosine and ascorbic acid. Hydrogen peroxide plays an important role in this reaction: the rates of formation of both dopa and 6-OH-dopa were increased by addition of hydrogen peroxide but diminished by addition of catalase. Methaemoglobin also catalysed the formation of cysteinyldopa from dopa and cysteine.

Key words: Methaemoglobin; Dopa; 6-OH-Dopa; 5-OH-Dopa; 5-S-Cysteinyldopa; Catalase; Hydrogen peroxide

The possible role of trihydroxy derivatives of phenylalanine in melanin biosynthesis has been investigated (3, 8, 9, 11–13, 15), and a methylated derivative of 6-hydroxydopa has been identified as a product of the microorganism *Microspira tyrosinatica* by direct comparison with an authentic sample (9). Recent work has demonstrated the formation of another trihydroxy compound, 5-OH-dopa, by mushroom tyrosinase (5, 6), and 5-OH-dopa has also been found to be a substrate of tyrosinase (1).

We now report the formation of 6-OH-dopa on incubation of tyrosine and ascorbic acid with an extract of bovine choroid–retinal pigment epithelium. Bovine retina and choroid were chosen because these melanin-containing tissues are readily available. It turned out however that the dominant catalytic activity of our extracts was not related to tyrosinase, but to haem pigment.

EXPERIMENTS AND RESULTS

Retina and choroidea from 50 bovine eyes (weight 33 g) obtained from a slaughter-house were dissected and kept on ice. Phosphate buffer, 0.01 M, pH 6.5, was added to 200 ml. The tissue was finely chopped with scissors, and homogenized with a glass homogenizer. The homogenate was kept overnight at −20°C. After thawing, it was treated with ultrasound for 20 min on ice, the temperature being kept below 5°C. Large particles of debris were separated by centrifugating at 200×*g* for 5 minutes, and the supernatant was centrifuged at 30 000×*g* for 10 minutes. Ammonium sulphate was added to the supernatant to 10% saturation, and after 1 hour at 0°C recentrifugation at 30 000×g for 1 hour was performed. There was very little sediment. Ammonium sulphate was added to the supernatant to 60% saturation. The precipitate was suspended in 50 ml 0.5 M phosphate buffer, pH 6.5.

The various sediments and supernatants were examined for dopa-oxidizing capacity. Dopa and cysteine were added to each sample to a final concentration of 10^{-3} M