

5-S-CYSTEINYLDOPA AND DOPA IN SERUM DURING TREATMENT WITH 8-METHOXYPSORALEN AND UVA LIGHT

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

Departments of Dermatology and Pharmacology, University of Lund, Sweden

Abstract. 5-S-Cysteinyldopa and dopa concentrations in serum were studied by high-performance liquid chromatography (HPLC) in patients with psoriasis treated by 8-methoxypsoralen and UVA light. A marked increase in 5-S-Cysteinyldopa was found after 3 days' treatment, although no increase in pigmentation could yet be observed. The highest concentrations of 5-S-Cysteinyldopa in serum were noted after 1 or 2 weeks' treatment. In one patient who showed no increase in serum 5-S-Cysteinyldopa treatment was unsuccessful. During PUVA treatment of 3 patients with psoriasis confined to the palms and/or soles a delayed increase in serum 5-S-Cysteinyldopa was noted after 5 weeks. This delayed response after treatment of a small area of the body is remarkable, and may indicate the formation of a systemic melanocyte stimulation factor. There was no increase in the serum dopa concentrations during PUVA treatment, and dopa analysis was of no value in assessing the activity of the melanocytes. Some unexpectedly high dopa values recorded before and during PUVA treatment may be explained by the fact that dopa originates in the nervous system or the adrenals.

Key words: 5-S-Cysteinyldopa; Dopa; 8-Methoxypsoralen; UVA light; Melanocytes

PUVA treatment leads to strong stimulation of pigmentation. An increased number of functionally active melanocytes has been reported (8, 9, 16), but this finding has not been confirmed by others (12). Melanosomes increase in number (8, 9, 15, 16), and increased tyrosinase activity in melanocytes has been observed after PUVA treatment (9).

Among the metabolites of the melanocytes dopa and 5-S-Cysteinyldopa (5-S-c) have a key role. Both are formed by the action of tyrosinase; dopa from tyrosine, and 5-S-c from dopa by oxidation to dopaquinone followed by nucleophilic addition of cysteine. Urinary excretion of 5-S-c gives a good idea of the serum concentrations of this amino acid, since it is not decarboxylated, but measurement of dopa excretion gives no definite picture of serum dopa concentrations because dopa is to a great extent decarboxylated and also further metabolized (2, 5).

We have previously studied the urinary excretion of 5-S-c in patients with psoriasis treated by 8-methoxypsoralen and UVA light (3). A marked increase took place after only 2 days' treatment, although no increase in pigmentation could yet be observed. Peak values for urinary 5-S-c excretion were noted after 1 or 2 weeks' treatment. Recently a sensitive method for quantitation of 5-S-c and dopa in serum has been described (4).

The aim of the present study was to investigate the chemical events in the melanocytes after PUVA treatment, as reflected by changes in the serum concentrations of 5-S-c and dopa. We also investigated whether PUVA treatment of only the palms and soles altered the serum concentrations of these amino acids.

MATERIAL AND METHODS

13 otherwise healthy psoriatic patients were chosen for the study. All received treatment with PUVA. 10 had widespread psoriasis; 3 had psoriasis of the palms and/or soles. 4 of the 10 patients with widespread psoriasis were studied for 8 days. Blood samples were collected before treatment, and then almost every day for the first 8 days of treatment. The other 6 patients were followed during 28 days' treatment. Blood samples were collected before treatment and after 1, 3, 7, 10, 14, 21, and 28 days' treatment.

The 3 patients with psoriasis of palms and soles (B. E. and A. H.) or soles only (I. S.) were followed during 30-40 days' treatment. Blood samples were collected before treatment and then at intervals of 5-7 days.

No patient was receiving any drug other than 8-methoxypsoralen. There had been no exposure to strong sunlight during the 2-3 months preceding the investigation. The investigation took place during the period October 1979 to March 1980 in order to avoid influence of sun exposure (10). All patients were given 20-60 mg of 8-methoxypsoralen according to body-weight, followed by irradiation after 2 hours; this procedure was carried out four times weekly (13). The light source was a high-intensity UVA system (PUVA 4000, Sylvania) with an emission spectrum between 320 and 390 nm and a peak emission of 365 nm. All 10 patients with widespread psoriasis

Table 1. Clinical and biochemical data in patients treated with PUVA

Patient	Age	Sex	Hair colour	Eye colour	Skin type ^a	MPD (J/cm ²) ^b	Initial PUVA dose (J/cm ²)	5-S-Cysteinyldopa (ng/ml)	
								Initial value	Max. value
R. H.	31	♂	Blond	Green	III	1.5	1	2.0	10
F. S.	68	♂	Brown	Brown	II	2	1.5	2.4	12
K. E.	28	♀	Blond	Blue	II	2	2	1.3	7.6
M. G.	50	♀	Blond	Green	II	3	2.5	1.7	12
A. H.	22	♂	Blond	Blue	II	3	2.5	2.7	21
C. N.	40	♂	Blond	Grey	III	3	2.5	4.3	44
A. P.	48	♂	Blond	Blue	II	4	2.5	2.2	6.5
L. C.	53	♂	Brown	Green	IV	3	3	1.3	6.2
C. S.	20	♀	Blond	Blue	III	7	3	2.9	4.1
K. K.	30	♂	Blond	Blue	III	5	3.5	1.3	14

^a The following criteria were used: Skin type I=always burn, never tan; II=always burn, then light tan; III=sometimes burn, always tan; IV=never burn, always tan.

^b MPD=the patient's minimum phototoxicity dose.

were phototested as described by Wolff et al. (14). The initial dose was in most cases well below the minimum phototoxic dose (MPD). The dose was as a rule increased once per week, and then by 0.5 J/cm² at each session.

The 3 patients with psoriasis localized to the palms and/or soles were treated three times a week, local irradiation being provided by a small PUVA unit (PUVA 180 combined with PUVA 200, Sylvania); the initial dose of UVA was 2.5 J/cm², and was increased by 0.5 J/cm² every second time (7).

10 ml venous blood samples for 5-S-c and dopa analysis were collected before and after varying intervals during

PUVA treatment into glass tubes containing 10 mg sodium metabisulphite. Serum was precipitated with 1/10 volume 4 M perchloric acid, centrifuged at 15000 r.p.m., and filtered. 5-S-c and dopa were adsorbed onto Al₂O₃ at pH 8.6, eluted with 0.6 M perchloric acid, and then determined by HPLC and electrochemical detection (4).

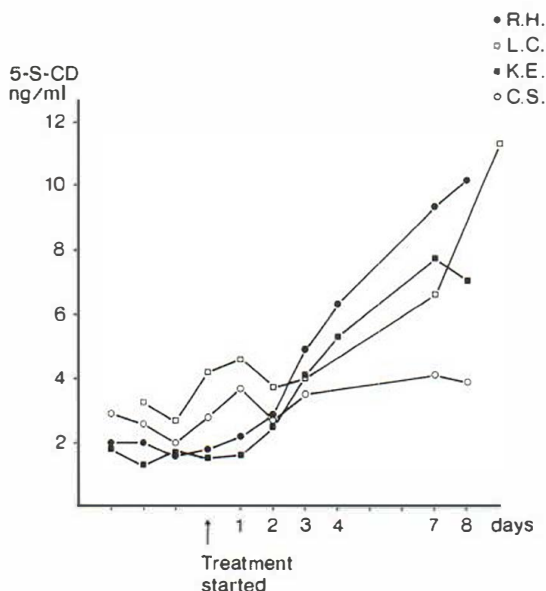


Fig. 1. Serum concentration of 5-S-cysteinyldopa during the first week of treatment.

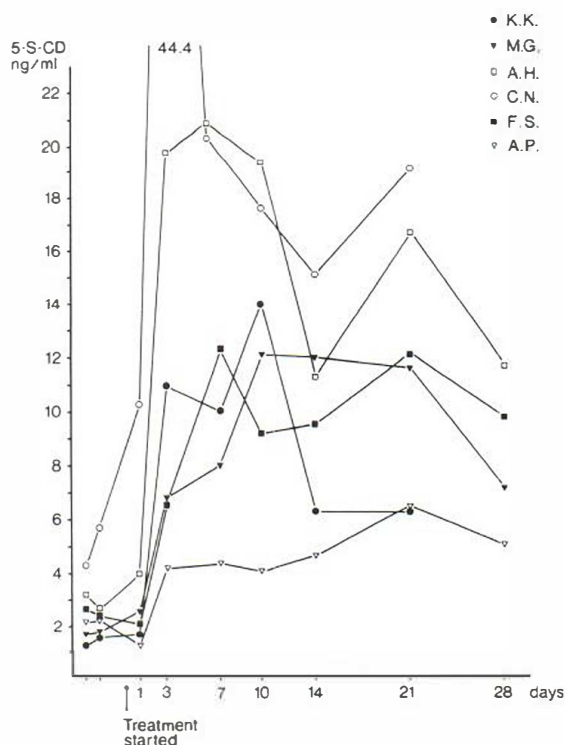


Fig. 2. Serum concentration of 5-S-cysteinyldopa during 4 weeks' treatment.

Table II. Serum concentrations (ng/ml) of free dopa during PUVA treatment

		Days after start of treatment							
Patient		Before treatment	1	3	7	10	14	21	28
K. K.	11	1.9	10	3.0	3.6	2.5	3.5	2.6	
M. G.	4.0	3.0	4.3	8.8	2.3	3.4	2.4	2.6	1.6
A. H.	1.2	1.4	1.8	1.7	2.6	3.4	2.2		
C. N.	2.5	2.1	2.5	2.8	2.5	2.7	2.9		
F. S.	2.5	1.9	2.2	2.2	2.4	2.8	3.3		
A. P.	1.9	1.6	25	7.0	1.8	1.8	3.2	2.2	1.7

RESULTS

5-S-Cysteinyldopa

Whole-body treatment. The concentration of 5-S-c in serum before treatment was between 1.3 and 4.3 ng/ml (Table I). These values were within the normal range (5). After 3 days 5-S-c values were increased in all but 2 patients (Figs. 1 and 2). At this time no increase in pigmentation could be seen, but such was first noted after 3–4 treatments. The maximum serum concentration was recorded after 4–6 treatments, i.e. after 7–10 days. In the patients with the highest observed values (Table I and Fig. 2) widespread erythema was present at the same time (C. N. and A. H.). The high values observed in some patients after 3 weeks' treatment were accompanied by localized erythema in 2 patients (C. N. and A. H.) or by very marked hyperpigmentation on the knees and medial malleoli in 1 patient (F. S.).

The psoriasis lesions cleared up in 9 of the 10 patients after 3–8 weeks' treatment. 8 of the 9 developed pronounced tanning. Pigmentation was most prominent in the previously affected skin areas. 2 patients (C. S. and A. P.) developed only a slight tan, and the increase in serum concentration of 5-S-c was small (Table I). In C. S. the psoriasis failed to clear up during PUVA treatment, which was discontinued after 30 sessions. After 18 sessions the serum concentration of 5-S-c was the same as before treatment. As the psoriasis did not respond to treatment the plasma concentration of 8-methoxypsoralen was measured 2 h after taking the drug (6). The 8-MOP plasma concentration was 0.60 $\mu\text{mol/l}$, which is within the normal range. In A. P. the psoriasis cleared up, but recurred only 2 weeks after stopping treatment.

Treatment of palms and soles. The serum concentration of 5-S-c increased also in the 3 patients

treated locally on the palms and/or soles (Fig. 3). Here the maximum concentration appeared later, after 11–14 sessions, i.e. 33–40 days after starting treatment. One (A. H.) showed an increase in serum 5-S-c after seven treatments, when erythema and slight oedema were present on the palms and soles. When the highest serum value was noted (26.3 ng/ml) the erythema and oedema had disappeared completely. The other 2 patients showed no erythema during the course of treatment. None developed perceptible pigmentation of the palms and soles. In 2 patients (I. S. and B. E.) the psoriasis had cleared completely after 13 and 11 sessions, respectively, and PUVA was discontinued. In the third (A. H.) healing occurred after a further month's treatment, but the serum 5-S-c concentration was not monitored during the last month.

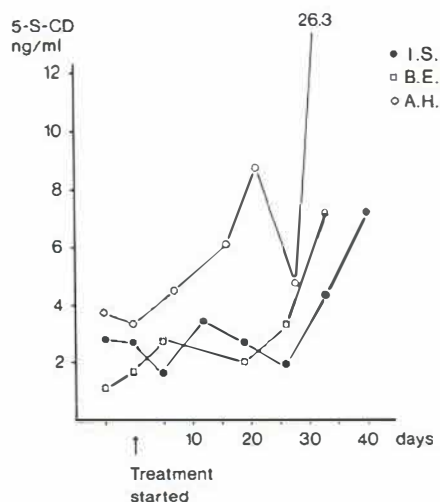


Fig. 3. Serum concentration of 5-S-cysteinyldopa in 3 patients irradiated on palms and/or soles.

Dopa

The great majority of the serum dopa values were within the normal range (5) and did not increase during PUVA treatment. In 3 patients (K. K., M. G., and A. P., Table II) high values were occasionally observed before or at the beginning of treatment, without correlation to clinical changes.

DISCUSSION

An increase in serum concentration of 5-S-c was found after only 3 days of treatment, before any detectable increase in pigmentation had been induced. Maximum serum concentrations were recorded after 4–6 sessions. These findings conform to those for urinary 5-S-c during PUVA treatment (3). The 2 patients showing the smallest increase in serum 5-S-c developed only a slight tan. One of them (C. S.) failed to respond to PUVA treatment, although the 8-MOP plasma level was within the normal range. This patient may have been undertreated. As she was fair-haired and blue-eyed an initial PUVA dose of 3 J/cm² was chosen, although the MPD was 7 J/cm². The doses of UVA were designed to give minimal erythema reaction during treatment. In 2 patients who developed pronounced erythema, very high values of 5-S-c were noted at the time of the erythematous reaction. This supports the hypothesis that increased 5-S-c excretion from the melanocytes is rather a reflection of damage to these cells than of pigment production (3). Absence of increase in 5-S-c or of pigment response is probably an indication for more intense treatment.

In the 3 patients given PUVA treatment to palms and/or soles, there was a delayed increase in the serum concentration of 5-S-c which appeared 33–40 days after treatment was started. The absorption and scattering of UVA light may have been so intense in the thick, diseased epidermis that the irradiation could not penetrate to the melanocytes until the stratum corneum had become normal. If this is so, the great increase in 5-S-c after treatment of a small area of the skin is remarkable.

Another explanation must also be considered. If the melanocytes were influenced by the treatment from the start, the number of activated melanocytes may have been too small to produce any detectable increase in serum 5-S-c. Activation of melanocytes outside the treated skin, such as recently described in mice (11), could then explain the delayed in-

crease in 5-S-c. Observations of elevated urinary excretion of 5-S-c in the spring (10) may also conform to the production of a systemically active melanocyte-stimulating factor that need not necessarily lead to increased pigmentation but to morphological and biochemical changes.

There was no increase in the serum dopa concentrations during PUVA treatment. We cannot explain the high dopa values in some of our patients: they could be of extramelanocytic origin, from the adrenals or the nervous system. In the biochemical diagnosis of melanoma metastases, 5-S-c determinations are of much greater value than dopa measurements (1). The present data on 5-S-c and dopa in connection with photochemically induced increase in pigmentation support the view that 5-S-c—and not dopa—is the marker of the pigment metabolism.

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REFERENCES

1. Agrup, G., Agrup, P., Andersson, T., Hafström, L., Hansson, C., Jacobsson, S., Jönsson, P.-E., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: 5 years' experience of 5-S-cysteinyl-dopa in melanoma diagnosis. *Acta Dermatovener (Stockholm)* 59: 381, 1979.
2. Agrup, G., Falck, B., Hansson, C., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Metabolism of 5-S-cysteinyl-dopa by O-methylation. *Acta Dermatovener (Stockholm)* 57: 309, 1977.
3. Agrup, G., Hansson, C., Rorsman, H., Rosengren, A.-M., Rosengren, E. & Tegner, E.: 5-S-Cysteinyl-dopa excretion after treatment with 8-methoxypsoralen and UVA light. *J Invest Dermatol* 70: 25, 1978.
4. Hansson, C., Agrup, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Analysis of cysteinyl-dopas, dopa, dopamine, noradrenaline and adrenaline in serum and urine using high-performance liquid chromatography and electrochemical detection. *J Chromatogr* 162: 7, 1979.
5. Hansson, C., Thysell, H., Lindholm, T., Agrup, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Dopa metabolism in uremia and RDT-patients. In *Proceedings of the European Society for Artificial Organs (ESAO)*, Vol. VI, Ed. E. S. Büchler, Geneva, Switzerland, 1979.
6. Ljunggren, B., Carter, M., Albert, J. & Reid, T.:

- Plasma levels of 8-methoxypsoralen determined by high-pressure liquid chromatography in psoriatic patients ingesting drug from two manufacturers. *J Invest Dermatol* 74: 59, 1980.
7. Morison, W. L., Parrish, J. A. & Fitzpatrick, T. B.: Oral methoxsalen photochemotherapy of recalcitrant dermatoses of the palms and soles. *Br J Dermatol* 99: 297, 1978.
 8. Pathak, M. A., Jimbow, K., Parrish, J. A., Kaidbey, K. H., Kligman, A. L. & Fitzpatrick, T. B.: Effect of UV-A, UV-B, and psoralen on in vivo human melanin pigmentation. *In Pigment Cell*, vol. 3 (ed. V. Riley), pp. 291-298. Karger, Basle, 1976.
 9. Pathak, M. A., Krämer, D. M. & Fitzpatrick, T. B.: Photobiology and photochemistry of furocoumarins (psoralens). *In Sunlight and Man*, (ed. T. B. Fitzpatrick), pp. 335-368. University of Tokyo Press, 1974.
 10. Rorsman, H., Agrup, G., Falck, B., Rosengren, A.-M. & Rosengren, E.: Exposure to sunlight and urinary excretion of 5-S-cysteinyl-dopa. *In Pigment Cell*, vol. 2 (ed. V. Riley), pp. 284-289. Karger, Basle, 1976.
 11. Rosdahl, I.: Local and systemic effects on the epidermal melanocyte population in UV-irradiated mouse skin. *J Invest Dermatol* 73: 306, 1979.
 12. Rosdahl, I. & Swanbeck, G.: Effects of PUVA on the epidermal melanocyte population in psoriatic patients. *Acta Dermatovener (Stockholm)* 60: 21, 1980.
 13. Wolff, K., Fitzpatrick, T. B., Parrish, J. A., Gschnait, F., Gilchrist, B., Hönigsmann, H., Pathak, M. A. & Tannenbaum, L.: Photochemotherapy for psoriasis with orally administered methoxsalen. *Arch Dermatol* 112: 943, 1976.
 14. Wolff, K., Gschnait, F., Hönigsmann, H., Konrad, K., Parrish, J. A. & Fitzpatrick, T. B.: Phototesting and dosimetry for photochemotherapy. *Br J Dermatol* 96: 1, 1977.
 15. Zaynoun, S., Konrad, K., Gschnait, F. & Wolff, K.: The pigmentary response to photochemotherapy. *Acta Dermatovener (Stockholm)* 57: 431, 1977.
 16. Zcllickson, A. S., Mottaz, J. H. & Muller, S. A.: Melanocyte changes following PUVA therapy. *J Am Acad Dermatol* 1: 422, 1979.

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E. Tegner, M. D.
Department of Dermatology
University Hospital
S-221 85 Lund, Sweden