Acta Dermatovener (Stockholm) 59: 149–151, 1979

LACK OF DERMAL CHANGES IN UNINVOLVED SKIN AFTER PHOTOCHEMOTHERAPY OF PSORIASIS: BIOCHEMICAL STUDIES

Nelly Blumenkrantz, Kaare Weismann and Gustav Asboe-Hansen

Department of Dermatology, University of Copenhagen, Rigshospital, Copenhagen, Denmark

Abstract. Low Hyp and Hyl values were found in psoriatic lesions. Treatment with PUVA achieved a return to normal values. No effect of PUVA on the collagen of uninvolved skin of psoriatic patients could be demonstrated after 4 months. There were no age-related differences in the content of Hyp and Hyl between samples of uninvolved skin of psoriatics.

It is known that skin collagen and elastin are affected by ultraviolet light (4, 6). According to Woessner (6), the condition of actinic elastosis involves a roughly 50% decrease in hydroxyproline. The introduction of photochemotherapy for psoriasis, a combination of oral intake of psoralen and irradiation with ultraviolet light having a wavelength of about 360 nm, has aroused interest in the possibility of introducing permanent changes of dermal fibres in the patients.

We undertook biochemical studies on skin, with particular reference to collagen before and after the mentioned treatment.

MATERIALS AND METHODS

Patients

Seventeen patients (3 males and 14 females) suffering from severe psoriasis were included in the study. Apart from one patient (No. 21, Table I), who had generalized guttate psoriasis, all suffered from long-standing recalcitrant psoriasis of the nummular or plaque type. The age range of the males was 46–69 years and that of the females 17–67 years. None of them had received psoralen photochemotherapy (PUVA) before.

Treatment schedule

All patients were started on PUVA according to a fixed schedule, using oral doses of 8-methoxy-psoralen (Meladinine®, The Memphis Chemical Co., Cairo, Egypt) of about 0.5 mg per kg body weight. The tablets were ingested 2 hours prior to UVA exposure from Philips TL/08 tube lamps with a spectral range of 320-400 nm. The UVA energy reaching the body surface of the patients was 4.5 mW/cm^2. The UVA dose was raised from 1.5 joule/cm^2 up to a maximum of 5 joule/cm^2 in stages of 0.5 joule/cm^2 at each subsequent exposure. The treatment was given three times weekly.

Skin biopsies

Punch biopsies were taken under local ethyl chloride surface anesthesia. Double biopsies were removed from the hip region of 15 patients, one from an affected area, the other from a non-affected area. Two patients had their biopsies taken on the back of the thigh (Nos. 4 and 6, Table I). The biopsies were systematically taken in the following periods: 1) before PUVA treatment was started; 2) after 4 weeks' treatment; 3) 4 months after starting the treatment. In case of complete clearing of psoriasis only one specimen was removed, from uninvolved skin.

A control biopsy of unexposed skin of one of the patients was taken together with those of involved and uninvolved areas exposed for one month. The total UVA radiation doses are given in Table I. Each patient served as his/her own control.

Biochemistry

The scales were cleaned off the skin before the biopsies were taken. Biochemical studies were performed on dry defatted samples. The biopsies were treated with 1) acetone, 3 changes per day for 3 days; 2) acetone : ether (1 : 1), 3 changes on 1 day; and 3) 1 change of ether. After the rest of the ether was evaporated, the samples were placed in an exsiccator under vacuum for 3 days. Scales were submitted to the same procedure. Samples were weighed in an ultramicro Mettler balance, and hydrolysed with 2 ml 6 N HCl at 118°C for 16 hours, after which they were centrifuged at 1 800 rpm for 10 min. Two aliquots were taken from the supernate, i.e. one of 0.1 ml and another of 1.5 ml, to be used for determination of hydroxyproline (Hyp) and hydroxylysine (Hyl). The HCl was evaporated under vacuum at 65°C, and the dried samples were dissolved in the corresponding buffers and assayed for Hyp and Hyl according to Blumenkrantz & Asboe-Hansen (1, 2).

The effect of the treatment on the content of Hyp and Hyl of normal uninvolved as well as of involved skin of psoriatic patients was studied. The same parameters were studied on uninvolved as well as involved skin of 36 psoriatic patients who received no treatment. The molar ratio of Hyp/Hyl was calculated in all samples (3). The

Table I

Patients no.	Accumulated PUVA dose at 2nd biopsy (after 4 weeks) (joules/cm²)	Maintenance PUVA dose at 3rd biopsy (after 4 months) (joules/cm²)
1	46	60
2	46	–
3	46	–
4	46	–
5	46	–
6	46	–
7	31[a]	–
8	46	–
9	46	60[c] –
10	46	–
11	46	–
12	46	–
13	46	–
14	46	180[d] –
15	46	–
16	46	60[c]
17	21[b]	–

[a,b] Complete clearing after 21 days and 17 days, respectively.
[c,d] Treatment once and three times weekly, respectively.

Wilcoxon test for pair differences was used to estimate the significance of the changes in Hyp and Hyl and their molar ratio, before and after treatment.

RESULTS

Thirteen out of 17 patients experienced a marked improvement during the first 4 weeks of the study and their treatment was therefore discontinued. The remaining 4 patients continued to receive therapy once or three times weekly because of incomplete cure or slight recurrence (Table I).

At the end of the study, no psoriatic lesions were visible in 7 patients.

Hydroxyproline and hydroxylysine content in untreated psoriatic lesions. In comparison with the content in unaffected skin, the lesions showed low values for Hyp. The same was true of Hyl, although the absolute decrease was less marked than that of Hyp (Table II). The Wilcoxon test for matched pairs showed the differences between the values of Hyp and Hyl in affected vs. unaffected areas to be highly significant ($p<0.001$).

Effect of PUVA on the hydroxyproline and hydroxylysine content in uninvolved skin of psoriatic patients. In the 7 patients who, after 4 months, were free of psoriasis no significant differences were found in Hyp and Hyl values in biopsies taken before vs. after the study (Table III).

Effect of PUVA on the hydroxyproline and hydroxylysine content in psoriasis lesions during treatment. A normalization Hyp and borderline variations of Hyl in biopsies from affected areas taken before and after 4 months' treatment were observed in the 7 patients who displayed no psoriasis at the end of the study (Table III).

Comparison of the content of Hyp and Hyl in control exposed and unexposed skin. The values from both biopsies were within the normal range. The Hyp and Hyl values were expressed as μg/10 mg DDS. (Control exposed: Hyp 839, Hyl 53.6, molar ratio 22.95; control unexposed: Hyp 903, Hyl 58.4, molar ratio 22.6.)

Scales. Very low Hyp and Hyl contents were found, viz. Hyp 29±4 μg/10 mg dry weight; Hyl 3.6±0.4 μg/10 mg; molar ratio Hyp/Hyl 10±2. These are average values ±S.D. of the analyses of scales from 3 patients.

Age differences. No significant differences in the content of the two amino acids studied were observed in the biopsies of uninvolved skin of patients aged from 17 to 67 years.

DISCUSSION

The relationship between the clinical improvement of the psoriatic lesion and the normalization of the values of Hyp and Hyl is worth noting. The low values of the two amino acids in the lesions before treatment and in the patients who did not respond to the PUVA treatment, is presumed to be due to a dilution effect brought about by a thickening of the epidermis and the inflammation in the dermis. Our results do not agree with those reported by Fleckman et al. (5), who found no difference in the content of Hyp in involved and uninvolved

Table II. *Average values of hydroxyproline and hydroxylysine in skin of 36 psoriatic patients who received no treatment*

Two biopsies were taken from each patient, one from a lesion (L), the other from a corresponding, non-affected control area (C)

No. of patients	Hydroxyproline		Hydroxylysine	
	C	L	C	L
36	822±88	695±105	58±8	46±8

Results are expressed as μg/10 mg DDS (dry defatted skin).

Table III. *Hydroxyproline and hydroxylysine in involved and uninvolved skin of psoriatic patients before and after 1 and 4 months of PUVA treatment*

	Involved skin				Uninvolved skin			
	No.	Hyp (μg/ 10 mg DDS)	Hyl (μg/ 10 mg DDS)	Molar ratio Hyp/Hyl	No.	Hyp (μg/ 10 mg DDS)	Hyl (μg/ 10 mg DDS)	Molar ratio Hyp/Hyl
Before PUVA	7	703± 68	49± 7	18.46±2.09	7	829±87	59± 7	18.21±1.62
1 mo. PUVA	7	733± 66	51± 5	19.03±2.04	7	803±46	55± 6	19.18±1.89
4 mo. PUVA	4	846±100	57±15	21.25±4.53	4	889±93	57.42±12	20.31±2.70
					3[a]	877±47	53.3±8	21.16±1.90

[a] Healed lesions.

psoriatic skin. The latter authors expressed their results in mg collagen providing 13% of Hyp in collagen. Converting their collagen values into Hyp, the values are 565 and 631 μg Hyp per 10 mg dry weight in uninvolved vs. involved skin, which are considerably lower than our average values, especially for uninvolved skin. The difference must be assumed to be due to different sensitivity of the methods used.

REFERENCES

1. Blumenkrantz, N. & Asboe-Hansen, G.: An automated procedure for quantiative determination of hydroxyproline. Clin Biochem *7*: 251–257, 1974.
2. Blumenkrantz, N. & Asboe-Hansen, G.: Automated quantitative assay for hydroxylysine in biological materials. Clin Biochem *8*: 177–183, 1975.
3. Blumenkrantz, N. & Asboe-Hansen, G.: Hydroxyproline to hydroxylysine molar ratio indicates collagen type. Acta Dermatovener (Stockholm) *58*: 111–115, 1978.
4. Danielsen, L. & Kobayasi, T.: Degeneration of dermal elastic fibres in relation to age and light exposure. Acta Dermatovener (Stockholm) *52*: 1–10, 1972.
5. Fleckman, P. H., Jeffrey, J. J. & Eisen, A. Z.: A sensitive microassay for prolyl hydroxylase: Activity in normal and psoriatic skin. J Invest Dermatol *60*: 46–52, 1973.
6. Woessner, J. F., Jr: Biological mechanism of collagen resorption. *In* Treatise on Collagen (ed. G. N. Ramachandran) vol. 2, p. 253. Academic Press, London and New York, 1968.

Received August 28, 1978

G. Asboe-Hansen, M.D.
Department of Dermatology
Rigshospital, Blegdamsvej 9
DK-2100 Copenhagen
Denmark