R. M.: Subcorneal pustular dermatosis. Acta Dermatovener (Stockholm) *45:* 203, 1965.
8. Schnitzler, L., Verret, J.-C., Schubert, B., Pouplard, L. & Simon, L.: Pustulose sous-cornée de Sneddon-Wilkinson avec acantholyse et myélome IgA. Ann Dermatol Venereol (Paris) *104:* 170, 1977.
9. Sneddon, I. B. & Wilkinson, D. S.: Subcorneal pustular dermatosis. Br J Dermatol *100:* 61, 1979.
10. Tagami, H. & Ofugi, S.: Characterization of a leucotactic factor derived from psoriatic scale. Br J Dermatol *97:* 509, 1977.

Blood Polyamine Levels in Psoriasis Patients Undergoing PUVA Treatment

Elsa Rosengren and Eva Tegner

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

Received February 13, 1981

Abstract. Blood spermidine and spermine concentrations were determined in 6 men with psoriasis, before, during, and after treatment with PUVA. Blood samples from 13 healthy men of similar age were used as controls. Psoriasis patients showed a significant increase in spermine concentration, which persisted throughout 4–8 weeks of therapy, during which the psoriasis lesions cleared. High spermine concentrations were also noted 1 month after stopping treatment, whilst the patients were still in remission. No significant change in spermidine concentration was seen.

Key words: Polyamines; Spermine; Spermidine; PUVA; 8-Methoxypsoralen; UVA-light

An association of polyamine biosynthesis and accumulation with rapid mammalian cell growth and proliferation has recently been shown. A potential connection between polyamines and the epidermal-cell hyperproliferation of psoriasis was first reported by Proctor et al. (6), who noticed elevated concentrations of spermidine and spermine in the blood of patients with psoriasis. Raised extracellular concentrations of polyamines have since been reported in blood (2) and urine (9) in this disorder, and increased concentrations of polyamines and increased activity of their biosynthetic enzymes have been reported in psoriatic skin (8). A few studies on polyamine metabolism in psoriasis patients undergoing treatment have been reported. Therapy including topical administration of anthralin, salicylic acid, tar baths, ultraviolet light, and corticosteroids (7, 8) has resulted in reduced cutaneous concentrations of putrescine, spermidine, and spermine, and in reduced urinary excretion of spermidine and spermine. No sequential analysis of polyamine metabolism in patients receiving treatment with 8-methoxypsoralen and UVA light (PUVA) has yet been published. We now present findings on the concentrations of blood polyamines in patients undergoing PUVA treatment.

MATERIAL AND METHODS

6 men with psoriasis but otherwise healthy were chosen for the study. All had generalized symmetrical psoriasis. They were receiving no drug other than 8-methoxypsoralen. There had been no exposure to strong sunlight during the 2–3 months preceding the investigation. The patients were given 8-methoxypsoralen, 30–60 mg related to body weight, followed by irradiation 2 hours later. This procedure was carried out four times weekly (11). 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 patients were phototested, and the initial dose was kept below the minimum phototoxic dose (MPD). As a rule the dose was increased weekly by 0.5 J/cm^2. Treatment was stopped when the psoriasis lesions had cleared. No maintenance treatment was given.

Blood samples were collected just before treatment, and once each week during the treatment period until the lesions had cleared. Further blood samples were collected 1 month after treatment was stopped. As controls, blood samples from 13 healthy men aged 22–60 years were used. 3.5 ml of whole blood was mixed with 0.5 ml of sulphosalicylic acid solution (800 mg sulphosalicylic acid plus 0.32 mg ethylene diamine tetra-acetic acid disodium salt per ml). The mixture was frozen and thawed three times. After centrifugation the extract was adjusted to a pH of 2.0–.25 and passed through a filter with a pore size of 0.22 μm (Millipore Corp., Bedford, Mass. 01730). Chromatographic separation of the amines in the extract was carried out on a column of Durrum DC 6 A (7.0×0.6 cm) using an automatic amino acid analyser (LKB-BIOCAL 3201). By step-wise elution with sodium citrate buffers the amines emerged distinctly separated. The method used in our laboratory is described elsewhere (5).

RESULTS

The psoriasis lesions cleared up in all patients after 4–8 weeks of treatment (Table I) and the patients were still in remission 1 month after stopping treatment. The polyamine contents of blood from patients and controls are shown in Table II and Fig. 1.

Table I. *Clinical data in 6 patients treated with PUVA*

Patient	Age	Skin type[a]	MPD (J/cm^2)[b]	Number of treatments	Dose UVA (J/cm^2) Total	Dose UVA (J/cm^2) Range
G. N.	45	III	3	28	112	2.5–5.5
P. P.	37	II	2	21	54	1.5–4
J. N.	24	III	3	22	59	2–4
J. P.	54	III	4	21	72	2.5–4.5
A. A.	27	III	5	16	44	2–3.5
B. F.	29	I	1	16	21	0.5–2

[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 phototoxity dose.

A significant elevation of spermine concentration was observed in the patients (Table II). It should be noted that, although the lesions cleared in every patient after 4–8 weeks of treatment, the blood spermine concentration remained high; in fact, the highest values were found 1 month after stopping treatment, when the patients were still in remission.

Regarding blood spermidine, no significant difference was observed betweeen patients and controls (Table II). As to putrescine, a small chromatographic peak appeared with the same retention value as for putrescine in some samples from controls as well as from psoriasis patients; in other words, the concentration of putrescine was at or below the detection limit of the method (<0.7 nmol/ml blood) in all samples, and could not be reliably quantified.

Table II. *Concentrations (nmoles/ml) of spermidine and spermine in blood of 13 healthy men (controls) and of 6 patients with psoriasis during PUVA treatment*

Means and the S.E. of mean are given; numbers of observations in parentheses. * $P<0.05$, ** $P<0.01$ versus controls

	Spermidine	Spermine
Controls	10.1±0.81 (13)	7.3±0.70 (13)
Psoriasis		
Before treatment	9.8±0.51 (6)	10.2±0.77 (6)*
1 week of treatment	10.7±0.76 (6)	10.5±0.77 (5)*
2 weeks of treatment	11.4±1.03 (6)	11.1±1.27 (6)*
3 weeks of treatment	11.9±1.01 (6)	11.0±1.19 (6)*
4 weeks of treatment	11.0±0.66 (6)	10.4±0.54 (6)*
5 weeks of treatment	11.6±0.34 (3)	9.4±0.90 (3)*
6 weeks of treatment	12.3±0.66 (4)	10.6±0.75 (4)*
4 weeks after treatment	11.1±0.90 (5)	12.0±1.16 (5)**

In every individual the spermidine and spermine concentrations appeared fairly stable throughout the observation period (Fig. 1). Thus individual fluctuations in the spermidine/spermine ratio were modest. On comparing spermidine and spermine levels in different persons, individual variations emerged. In 2 patients (G. N. and A. A.) the spermidine level was higher than the spermine level, and the spermidine/spermine ratio was high (mean values 1.37±0.12 and 1.36±0.19 respectively). In another (B. F.) this relation was inverted, i.e. low spermidine/spermine ratio (mean 0.67±0.09) as the concentration of spermine was higher than that of spermidine. Finally, 3 patients showed ratios of 1.06±0.12, 1.01±0.10, and 0.97±0.16, reflecting a spermidine concentration of the same order of magnitude as that of spermine.

DISCUSSION

Changes in polyamine metabolism in skin, blood, plasma, and urine have been reported in patients with psoriasis (2, 6, 8, 9). Concerning blood polyamine concentrations in psoriasis, Cooper et al. (2) have summarized their observations on 14 patients: the blood spermine level was elevated, whereas the spermidine level was apparently unaltered. Our findings accord with their report.

Treatment of psoriasis lesions by various methods has been reported to result in decreased polyamine content of skin and urine (7, 8). In our patients the lesions began to clear after about 1

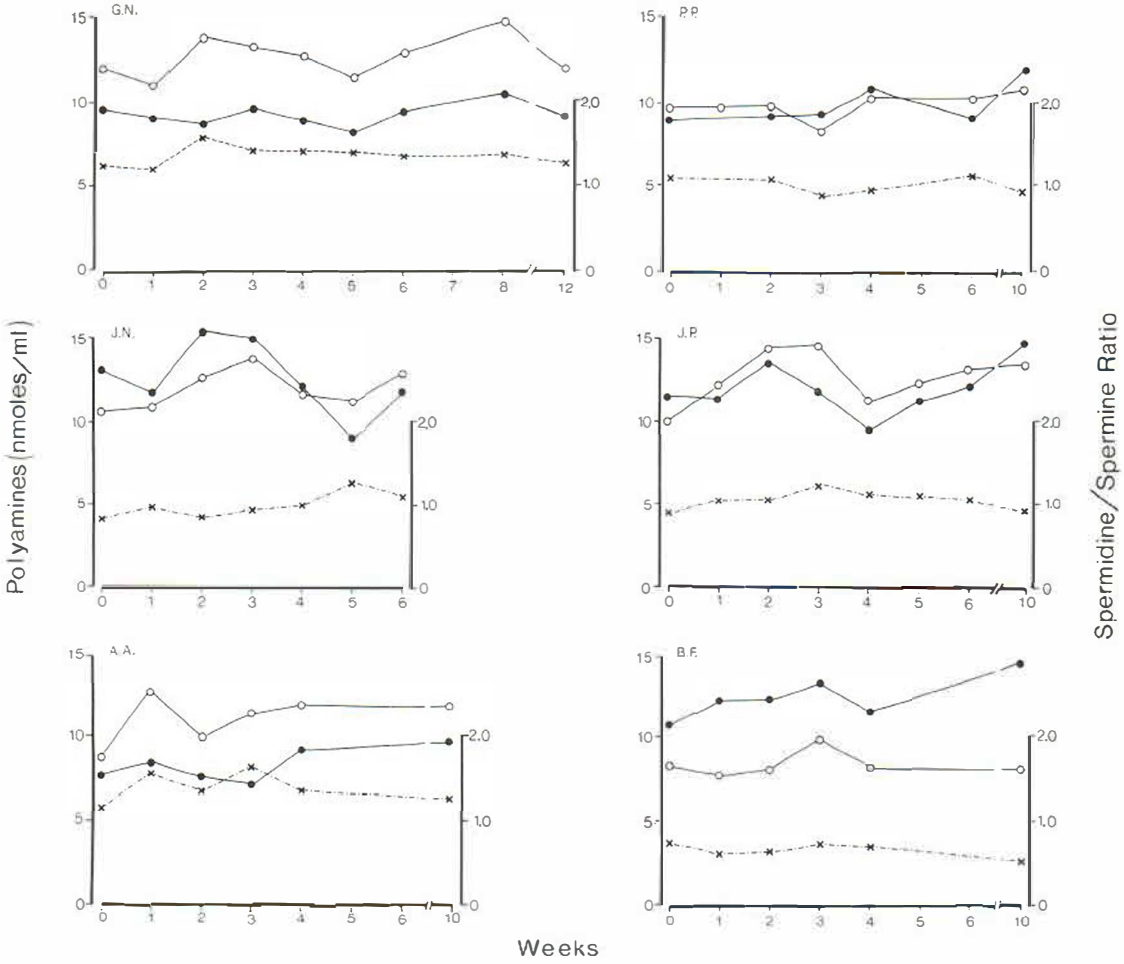

Fig. 1. Concentrations (nmoles/ml) of spermidine (○—○) and spermine (●—●) and the spermidine/spermine ratio (x---x) in the blood of 6 psoriasis patients before, during, and one month after PUVA therapy.

week of treatment, after which there was continued improvement until the psoriasis had cleared completely, when PUVA treatment was stopped. It is striking that the blood spermine concentration remained high for weeks, not only during therapy but also a month after treatment had been stopped. At this time all the patients were still in remission.

Irradiation of hairless mice with ultraviolet light of mainly the sunburn region (UVB) causes a dramatic increase in the activity of skin ornithine decarboxylase (10). This enzyme is considered to be the rate-limiting factor in the biosynthesis of polyamines. Maximum activity occurred at about 28 h, after which it declined to normal about 48 h after irradiation. In hairless mice topical application of 8-methoxypsoralen plus irradiation with long-wave ultraviolet light (UVA) at 3.6 J/cm^2 resulted in an increase in skin ornithine decarboxylase activity 16 to 72 h later (3). If the effect of PUVA treatment is analogous to this, a temporary increase in polyamine concentration in skin and blood might be expected. Any such effect ought, however, to have faded, and is unlikely to account for the high levels of spermine observed 1 month after treatment was stopped.

When haemolysis is completely prevented, the concentration of polyamines in plasma is low, and almost all the circulating polyamines are found in the blood cells (unpublished results). No significant differences have been found between the means for erythrocyte counts, total white cell counts, and differential cell counts in psoriatic patients and con-

trols (4). On a cell-to-cell basis, leukocytes contain more polyamines than do erythrocytes (1). Nevertheless, owing to the dominance of the erythrocytes, the bulk of blood polyamines are associated with these cells when calculated for 1 ml whole blood. Concerning the separate amines, the concentration of spermine in leukocytes is about twice that of spermidine. It is known that the polyamine content of erythrocytes declines with the age of the cell (1). The polyamine concentration as related to age of the leukocytes remains to be examined. Further work is required to elucidate the blood polyamines in psoriasis.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (No. B81-04X-02212-14A), from the Thure Carlsson Foundation for Scientific Research, and from the Åke Wiberg Foundation.

REFERENCES

1. Cooper, K. D., Shukla, J. B. & Rennert, O. M.: Polyamine distribution in cellular compartments of blood and in aging erythrocytes. Clin Chim Acta *73:* 71, 1976.
2. — Polyamine compartmentalization in various human disease states. Clin Chim Acta *82:* 1, 1978.
3. Gange, R. W. & DeQuoy, P.: Ornithine decarboxylase and DNA synthesis in mouse skin treated with PUVA or anthracene + UVA. Clin Res *28:* 134 A, 1980.
4. Hellgren, L.: Psoriasis: A statistical, clinical and laboratory investigation of 255 psoriatics and matched healthy controls. Acta Dermatovener (Stockholm) *44:* 191, 1964.
5. Henningsson, S.: Automated liquid chromatography in research on histamine metabolism. Agents Actions *8:* 392, 1978.
6. Proctor, M. S., Fletcher, H. V., Shukla, J. B. & Rennert, O. M.: Elevated spermidine and spermine levels in the blood of psoriasis patients. J Invest Dermatol *65:* 409, 1975.
7. Proctor, M. S., Wilkinson, D. I., Orenberg, E. K. & Farber, E. M.: Lowered cutaneous and urinary levels of polyamines with clinical improvement in treated psoriasis. Arch Dermatol *115:* 945, 1979.
8. Russell, D. H., Combest, W. L., Duell, E. A., Stawiski, M. A., Anderson, T. F. & Voorhees, J. J.: Glucocorticoid inhibits elevated polyamine biosynthesis in psoriasis. J Invest Dermatol *71:* 177, 1978.
9. Russell, D. H., Stawiski, M. A., Duell, E. A. & Voorhees, J. J.: Elevated polyamine metabolism in patients with psoriasis. Pharmacologist *18:* 157, 1976.
10. Verma, A. K., Lowe, N. J. & Boutwell, R. K.: Induction of mouse epidermal ornithine decarboxylase activity and DNA synthesis by ultraviolet light. Cancer Res *39:* 1035, 1979.
11. Wolff, K., Fitzpatrick, T. B., Parrish, J. A., Gschnait, F., Gilchrest, B., Hönigsmann, H., Pathak, M. A. & Tannenbaum, L.: Photochemotherapy for psoriasis with orally administered methoxsalen. Arch Dermatol *112:* 943, 1976.

Immunopotentiation of Allergic Contact Dermatitis in the Guinea Pig with *C. parvum* (*P. acnes*)

Henry C. Maguire, Jr

Department of Dermatology and Department of Microbiology and Immunology, Hahnemann Medical College, Philadelphia, PA 19102, USA

Received February 11, 1981

Abstract. An aqueous suspension of killed *C. parvum* (*P. acnes*) substantially immunopotentiates the acquisition of allergic contact dermatitis to low molecular weight allergens in the guinea pig. Damage to the skin from the adjuvant is slight.

Key words: Dermatitis, contact; Guinea pig; Dinitrochlorobenzene; Propionibacterium acnes; Adjuvant, immunologic

The guinea pig is the animal of choice for the prospective testing of low molecular weight chemicals that might be significant contact allergens in man (3, 4). A number of techniques are used in such testing to render the guinea pig more susceptible to contact sensitization. Magnification of immunological reactivity is necessary for the identification of weak contact sensitizers since under ordinary conditions such allergens have a low incidence of sensitization (viz. one per thousand or less). The use of complete Freund's adjuvant (CFA) is a particularly decisive way to intensify the induction of delayed-type hypersensitivity to simple chemical allergens in the guinea pig. However, CFA as an immunological adjuvant has the disadvantage that local draining inflammatory reactions form at the sites of CFA injections, and migratory granulomas, in the skin and elsewhere, often develop after the injection of CFA (1).