

SHORT REPORTS

Endothelial Cell Renewal in Skin Of Patients with Progressive Systemic Sclerosis (PSS): An *in vitro* Autoradiographic Study

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Received December 14, 1981

Abstract. The labelling index of endothelial cells of the dermal microvessels was studied by *in vitro* autoradiography with [³H] thymidine. 14 patients with PSS were compared with 7 control subjects, by sampling the upper-third of the forearm skin. One patient had a second biopsy in this area, on a telangiectasis. Microvessels from patients showed a significant increase in endothelial cell labelling. Within the PSS group, no difference was found between the indurated and the non-indurated skin. There was no correlation between the labelling index and the duration of the disease. A very high uptake was found in the telangiectasis. Such an increased labelling index in PSS is consistent with a response to the endothelial cell destruction as described in electron microscopy and in cultures with patient's serum.

Key words: Scleroderma; Autoradiography; Microangiopathy; Endothelial cell

Small blood vessel involvement has been emphasized as a nearly constant feature in progressive systemic sclerosis (PSS). Although the structural basis of this microangiopathy is not completely clear, evidence for endothelial injury and repair has been supported by ultrastructural observations (3, 12, 16). These are: (a) a marked reduction in capillaries; (b) degenerative changes in endothelial cells; (c) layering of capillary basal lamina which is known to result from an accelerated rate of cell death and replacement in other conditions (17).

In 1977, Fleishmajer & Perlish studied the *in vitro*

uptake of [³H] thymidine by endothelial cells of the skin and showed a marked increase in the endothelial cell labelling index (4, 5). In their series, the endothelial cell renewal was greater in clinically involved areas than in uninvolved ones. In addition, the labelling index was low in telangiectasias. These findings are at variance with the multisystemic involvement in PSS (2, 6) and the presence in serum of a selective cytotoxic activity, specific for endothelial cells (10).

The present study was undertaken 1) to investigate the *in vitro* [³H] thymidine uptake in skin biopsies obtained from patients with PSS and age-matched controls; 2) to correlate endothelial cell renewal and clinico-histological skin involvement.

MATERIAL AND METHODS

Patients

Fourteen patients, all females, were included in the PSS group. Their ages varied from 44 to 86, with an average of 62.7 ± 13.3. The criteria for the diagnosis were: Raynaud's phenomenon, abnormal thickening and tightness of the skin extending beyond the fingers, capillary changes as seen by nailfold capillaroscopy and/or visceral involvement; in this group, 10 patients had acrosclerosis and 4 met the criteria of the CREST syndrome, as previously defined (14). None of them exhibited diffuse scleroderma.

Six had undergone long-term treatment with colchicine (1-3 mg per day) and the other 8 received only a symptomatic treatment for their oesophageal involvement (cimetidine, aluminium hydroxide). The duration of the disease ranged from 3 to 58 years. Clinical information for each case is reported in Table I.

The controls were 7 patients (5 females, 2 males; average age 56.4 ± 18) with venous insufficiency of the lower limbs. None of them exhibited clinical or laboratory evidence of connective tissue disease.

Tissue sampling

All skin specimens were obtained about 1 p.m. with a 2-mm punch under local anesthesia with 1% xylocaine without adrenalin, from the upper third of the anterior aspect of the forearm. In 7 cases, the investigated area was clinically indurated. In the other 7 cases, the area appeared normal-looking and supple; light microscopy revealed no dermal fibrosis. In one patient, two biopsies were performed simultaneously in this area, the second specimen being taken from a telangiectasis.

The biopsy samples were minced immediately into 1 mm blocks and placed in Hanks' medium.

Table 1. *Clinical information*

Case no.	Sex	Age (years)	Raynaud's phen.	Duration of disease (years) ^a	PSS type	Gastro-oesophageal involvement	Lung involvement	Sclerosis of biopsy site	Colchicine
1	♀	71	+	25	Acrosclerosis	+	+	+	+
2	♀	62	+	4	Acrosclerosis	+	+	+	+
3	♀	50	+	20	Acrosclerosis	+	+	+	-
4	♀	45	+	5	Acrosclerosis	-	+	+	-
5	♀	86	+	10	Acrosclerosis	+	+	-	+
6	♀	46	+	30	CREST	+	+	-	-
7	♀	73	+	3	Acrosclerosis	+	+	-	-
8	♀	56	+	15	Acrosclerosis	+	+	-	-
9	♀	70	+	58	CREST	-	+	-	-
10	♀	59	+	15	CREST	+	-	-	+
11	♀	68	+	32	Acrosclerosis	+	+	+	+
12	♀	69	+	5	Acrosclerosis	+	+	-	-
13	♀	80	+	66	CREST	+	+	+	-
14	♀	44	+	20	Acrosclerosis	-	+	+	+

^a We have considered that the onset of the PSS dated since the appearance of the Raynaud's phenomenon.

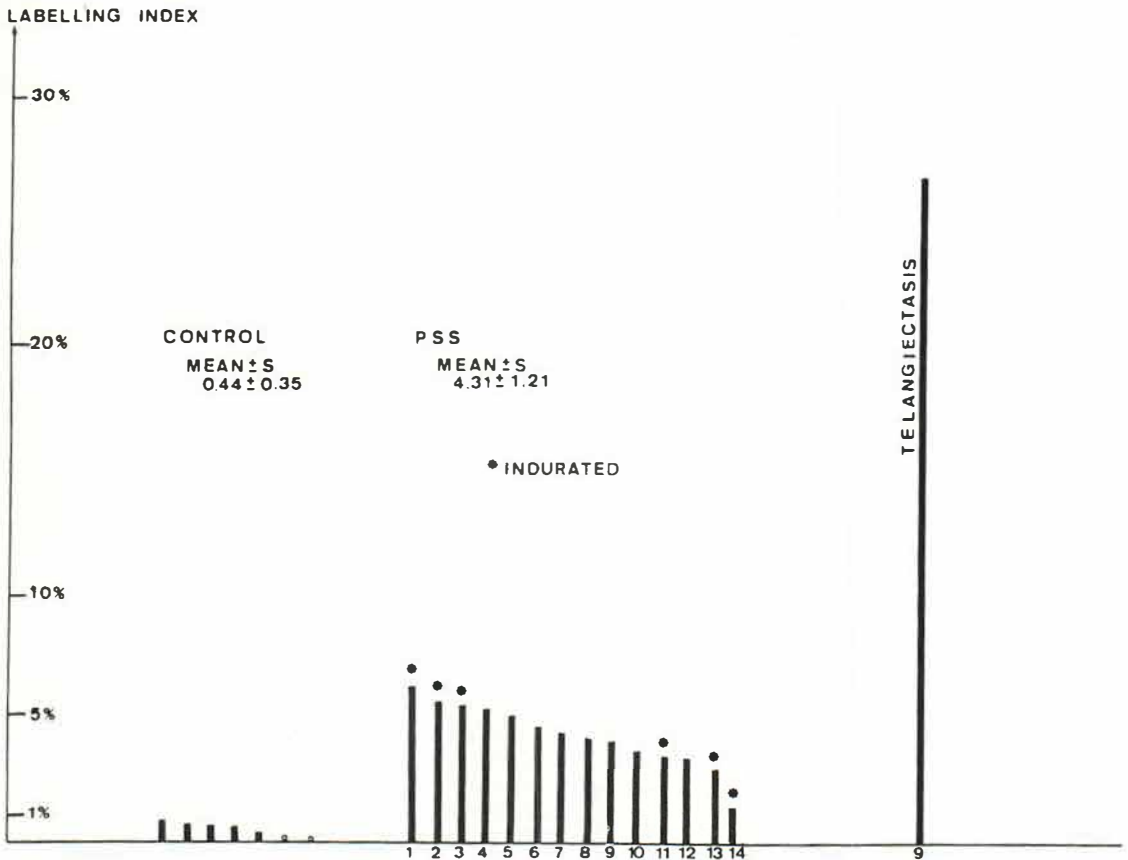


Fig. 1. Labelling index findings.

Autoradiographic study

The specimens were immersed in M.E.M. (Minimal Essential Medium), enriched with 12% fetal calf serum, and incubated at 37°C in a shaking water bath, in an atmosphere of 5% CO₂ and 95% O₂.

The sample was incubated in the medium for 1 hour; then in the medium with methyl[³H]thymidine, 10 μCi/ml, for 1 hour (specific activity: 25 Ci/mmol; Commissariat à l'Énergie Atomique); and in the medium alone for 1 hour again. Following these 3 hours of incubation, the sample was fixed in Bouin's fluid, dehydrated in alcohols, embedded in paraffin wax and the entire specimen was serially sectioned (5 μm each).

After removal of paraffin wax, prestaining with PAS and Harris hematoxylin, the slides were dipped in Ilford K5 nuclear emulsion diluted 1:1 in distilled water, under an atmosphere at 28°C and 90% hygrometry. The slides were exposed for 8 days at 4°C in light-tight boxes and then developed in Kodak D19B, and finally mounted before microscopic examination. The background count was sufficiently low (<2 grains/cell) to permit the easy identification of labelled cells. Cells with more than 10 silver grains over their nucleus were classed as labelled. However, most of labelled cells had so many grains that the nucleus appeared uniformly black. The labelling index determination was concentrated on the endothelial cells surrounding the lumen of dermal blood microvessels, red-rimmed by the PAS. Cell counts were performed on at least every sixth histologic section, the entire sample being studied. In each case, the labelling index was estimated by the ratio of labelled endothelial cells and the total number of endothelial cells, multiplied by 100.

Statistics

The distribution-free Mann-Whitney test was used for statistical significance assessment, because of the wide distribution of variances ($F_{13, 6} = 12.16$; $\epsilon_{\mu} = 3.65$).

RESULTS

In the PSS group, the labelling index of endothelial cells was significantly increased as compared with the control group ($p < 0.0001$) (Figs. 1, 2).

On the other hand, there was no difference between skin specimens obtained from clinically indurated areas ($m = 4.41\%$, $s = \pm 1.69\%$, $n = 7$) and those obtained from clinically and histologically normal areas ($m = 4.21\%$, $s = \pm 0.53\%$, $n = 7$). Nor was there any difference with regard to the treatment; the patients treated with colchicine had a labelling index in the same range as the others.

In the patient from whom two specimens were obtained, the telangiectasis was found to have a much higher labelling index (27%) than the other investigated site (4%) (Figs. 1, 3). No relation was found between the labelling index and the duration of the disease ($r = 0.30$; $t = 1.09$ N.S.).

DISCUSSION

The 7 subjects of the control group had a labelling index comparable to the normal values in the literature (4, 8, 15).

In the present study, the increase in the labelling index of the skin microvessel endothelial cells can be confirmed. Our data for clinically altered skin areas are in good agreement with those of Fleishmajer & Perlish (5). However, these authors found in 4 patients having had simultaneous skin biopsies taken from indurated and non-indurated areas, a greater index in the indurated ones. In our series, there was no significant difference between the samples taken from indurated areas and those from normal-looking and non-indurated ones. This discrepancy could be due to differences in tissue sampling. Particularly, in our series, all skin biopsies were taken from the same area. The lack of linkage between skin sclerosis and labelling index of dermal microvessels agrees with the fact of a widespread microangiopathy in PSS patients (3), and the isolation in serum of a cytotoxic activity, specific for endothelial cells (10).

Our study did not reveal any difference due to colchicine therapy. Whereas colchicine has proved to have a certain efficacy in long-term treatment of PSS (1, 9), its activity does not seem to be linked to changes in cell kinetics.

Two main explanations for the increase in labelling index of dermal endothelial cells seem plausible 1) active angiogenesis, 2) acceleration of cell turnover following exaggerated cell death. Such a destruction of dermal microvessels is supported by the *in vivo* and ultrastructural observations of the microvasculature (3, 11, 12, 13, 16).

The increased uptake of [³H]thymidine might take place in order to replace damaged endothelial cells. When human endothelial cells are in culture, they exhibit a stringent pattern of growth, since they are able to synthesize DNA only when subconfluent. In a confluent monolayer, endothelial cells proliferate again only in response to the disruption of this layer (7). Endothelial cell proliferation does occur *in vivo*, however, whenever the endothelial lining is altered. In PSS, the etiology of microvascular damage is poorly understood, and it is important to identify the factors at work.

Of interest is the observation concerning the high [³H]thymidine uptake of endothelial cells in one telangiectatic lesion. In similar lesions, Fleishmajer

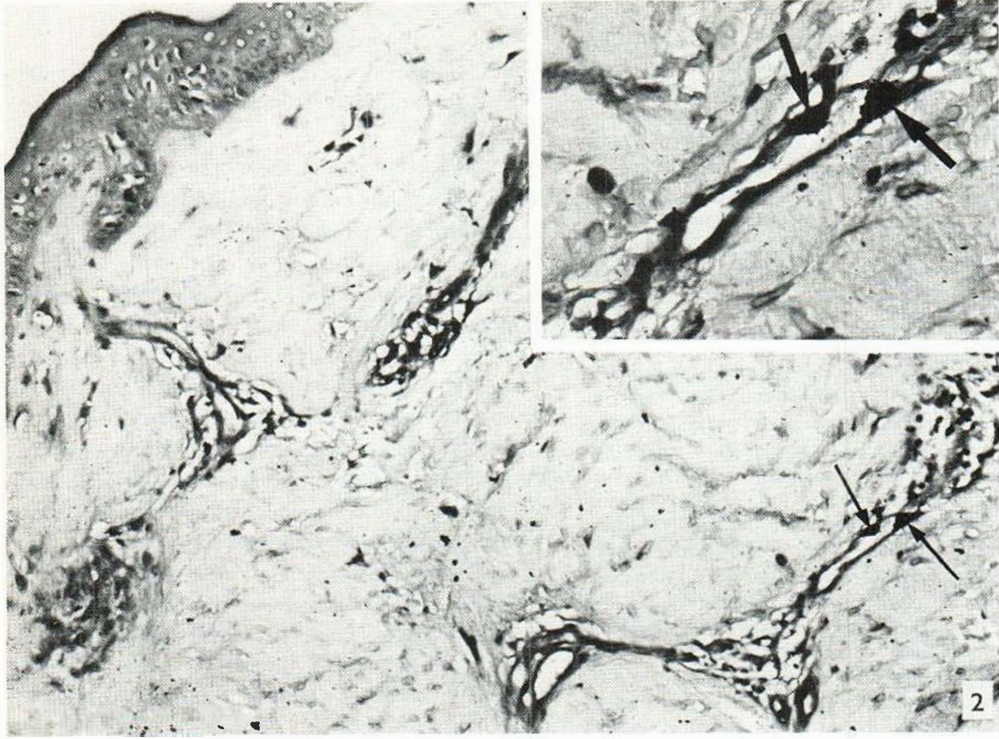


Fig. 2. PSS: two labelled endothelial cells (→) in the lower dermis ($\times 200$). *Inset*: same vessel at higher magnification ($\times 465$).



Fig. 3. PSS: several labelled endothelial cells in a telangiectatic vessel ($\times 250$).

& Perlish noted a very moderate increase in the labelling index. Such a discrepancy could be due to the fact that the entire sampling in our case consisted of an angioma-like telangiectasis and that the labelling index determination was restricted to the ectatic microvessels.

ACKNOWLEDGEMENT

The authors wish to thank Mrs Micheline Descarpentri for her helpful technical assistance.

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5% Aluminium Chloride Hexahydrate and Sebum Excretion Rate

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Received January 27, 1982

Abstract. The present study was designed to assess whether 5% aluminium chloride hexahydrate in absolute alcohol exerted any effect on sebaceous gland excretory activity. Eleven acne patients controlled with long-term antibiotics applied active and placebo solutions in a double-blind trial. The sebum excretion rate was measured on two control visits and thereafter at fortnightly intervals for a further 6 weeks. Patients preferred the 'active' solution, many claiming increased dryness of their skin. However, neither the 'active' nor 'placebo' solution had a significant demonstrable effect on sebum excretion rate. The beneficial effect of topical aluminium chloride hexahydrate is therefore assumed to be due to factors other than an alteration in sebum excretion.

Key words: Aluminium chloride hexahydrate; Sebum excretion rate

Acne is a multifactorial disorder involving increased sebaceous gland activity, pilosebaceous duct obstruction and abnormal microbial flora. Drugs which improve acne are likely to alter some or all of these factors. A 6.25% solution of aluminium chloride hexahydrate in absolute alcohol has been shown to improve acne (4). These authors demonstrated a reduction in sweat production and a decrease in the skin microflora and postulated that the clinical response obtained was attributable to these factors.