

Analysis of Elastin Metabolism in Patients with Late-onset Focal Dermal Elastosis

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Late-onset focal dermal elastosis has recently been described as new clinical entity characterized by pseudoxanthoma elasticum-like eruptions and an accumulation of normal-appearing elastic fibres in the dermis. Elastin and collagen contents of the skin of 2 patients were 2- and 1.4-fold higher than in the skin of controls, respectively. A focal accumulation of elastin but not of fibrillin-1 was observed by immunohistochemical staining. The levels of type I and III collagen and elastin mRNAs isolated from cultured patient fibroblasts were elevated 2–3-fold compared with control fibroblasts. There was no significant change in the excretion of elastin peptides in the urine of patients and controls. These results suggest that the focal accumulation of elastic fibres in the patient skin may be related to overexpression of elastin rather than to altered degradation of elastin. Key words: elastosis; pseudoxanthoma elasticum; collagen.

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We recently described 2 patients with lesions clinically resembling pseudoxanthoma elasticum and histologically exhibiting focal dermal elastosis, and proposed the term “late-onset focal dermal elastosis” as a new clinical entity. This disorder occurs preferentially in elderly people or in cases of premature ageing syndrome (1), suggesting that it is related to the ageing process. The pathogenesis of this disease is unknown at present. We studied the mechanism of the accumulation of elastic fibres in cultured skin fibroblasts established from the lesions of the 2 patients.

MATERIALS AND METHODS

Patients and fibroblast culture

Two patients with late-onset focal dermal elastosis were previously described in detail (2). Biopsies were taken from isolated papules in the groins using a 3-mm punch, taking care to exclude the normal region as much as possible. Fibroblasts were explanted from the skin biopsy samples of 2 patients and 4 age-, sex-, and site-matched controls. Cells grown in 35-mm Petri dishes in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS) were routinely subcultured every 6 days. Cells in passages 4–5 were used in this study.

Collagen and elastin contents in skin

Skin specimens were taken from 2 patients and 4 age-, sex- and site-matched normal individuals. The samples were hydrolysed with 6 M HCl for 18 h at 110°C. Hydroxyproline content was determined

according to the method of Prockop & Udenfriend (3). Isodesmosine content was determined with HPLC (LiChrosorb RP-18, Merck) (4).

Immunohistochemical staining

Skin specimens were embedded in OCT compound and cut into 5 µm sections. The sections were washed with PBS and fixed with acetone, then pre-treated with 2 M guanidine hydrochloride/0.1 M iodoacetamide for 30 min to expose the antigenic sites of target proteins (5). The sections were washed with PBS for 10 min, then treated for 5 min with 3% H₂O₂/PBS to inactivate endogenous peroxidase. The sections were incubated with 1 µg/ml of monoclonal antibody for bovine α-elastin (Sigma) or polyclonal human fibrillin-1 for 30 min at room temperature (6), then incubated with biotin-conjugated anti-mouse (1.4 µg/ml) or anti-rabbit (1.9 µg/ml) immunoglobulins (Dako) at 1:1000 dilution for 30 min at room temperature. The antigen–antibody complex was developed with avidin (10 µg/ml) and biotinylated peroxidase (2.5 µg/ml) for 5 min at room temperature (Dako) and 3-amino-9-ethyl carbazole (AEC) (0.44 µg/ml) (Dako) as enzyme and substrate, respectively.

Northern blot analysis

Total RNA was isolated from 2 patients and 4 age-matched (64–82 y-old) and site-matched (groin) control fibroblasts using a procedure described previously (7). RNA samples were denatured in deionized 1 M glyoxal/10 mM phosphate buffer, pH 6.5 at 50°C for 1 h, then resolved on 1% agarose gel electrophoresis and blotted onto nitrocellulose filters. RNA was immobilized on the filters by heating at 80°C for 2 h and hybridized to ³²P-labelled probes in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 0.1% SDS, 250 µg/ml sonicated salmon sperm DNA at 42°C for 18 h. The following cDNA probes, radioactively labelled by random priming, were used: pro α₁ (I) (Hf32) (8), pro α₂ (I) (Hf677) (9), pro α₁ (III) (Hf934) (10), elastin (pCHEL-2) (11) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (12) cDNAs. The filters were washed at a stringency of 0.1 × SSC at 42°C for 1 h and exposed to X-ray film at –80°C with an intensifying screen. The autoradiograms were scanned with a densitometer.

Measurement of elastin peptides in urine

Approximately 20 ml morning urine was collected from 2 patients and 5 normal healthy controls, and stored at –80°C until use. The amount of elastin peptides was determined by direct-binding ELISA using an anti-elastin peptide antibody (13, 14). Urinary creatinine was determined as previously described (15).

RESULTS

Collagen content, as determined by hydroxyproline content, of the patients' skin was slightly higher (1.4-fold) than that of the controls' skin (mean 63.2 vs 46.1). In contrast, elastin content, as determined by isodesmosine content of the patients' skin, was significantly elevated (approximately 2-fold) compared with that of the controls' skin (mean 15.3 vs 7.4) (Table I).

Immunohistochemical studies demonstrated that elastin was accumulated in the focal lesion of the patients' skin but no

Table I. Determination of collagen and elastin content in skin

Case	Age/sex	Hydroxyproline ($\mu\text{g}/\text{mg}$ dry skin)	Isodesmosine (nmol/mg dry skin)
Patient 1	85/M	57.4	12.2
Patient 2	65/M	69.0	18.4
Control 1	58/F	38.2	5.4
Control 2	56/M	45.1	7.1
Control 3	56/F	52.2	9.5
Control 4	68/M	49.0	7.7

significant accumulation of fibrillin-1 was observed (Fig. 1a and b).

RNA hybridization assays demonstrated that α_1 (I), α_2 (I) and α_1 (III) collagen chain and elastin mRNA levels in the patient fibroblasts were higher than in controls, whereas the GAPDH mRNA level was unchanged (Fig. 2a). Quantitative determination revealed that type I and III collagen and elastin expression in the patient fibroblasts was elevated 3.0-, 2.3- and 2.1-fold, respectively (Fig. 2b).

The excretion of elastin peptides in urine was determined. There was no significant change in the excretion of elastin peptides between patients and controls (Table II), suggesting that the degradation of elastin in the patient body was unchanged.

DISCUSSION

We have demonstrated the accumulation of elastin in the lesional skin of the patients by immunohistochemical staining. The result was consistent with our previous report showing the accumulation of van Gieson positive fibres (1). This was confirmed by biochemical data that isodesmosine content, a biochemical marker of elastin, was elevated in the patients' skin. It is noted that accumulation of fibrillin-1 was not found by immunohistochemical study in the focal lesion. Elastic fibres on electron microscopy consist of structurally different components, amorphous elastin and microfibrils. Fibrillin-1 is a major protein comprising microfibrillar components, which is

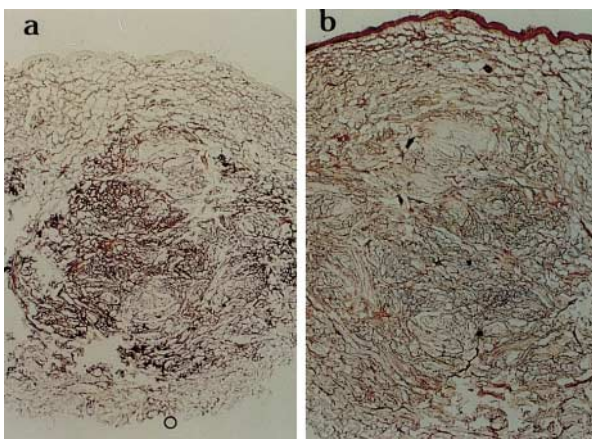


Fig. 1. Immunohistochemistry of elastin and fibrillin-1 in case 2 patient skin. The frozen skin specimen was fixed with acetone and stained with (a) anti-elastin or (b) anti-fibrillin-1 antibody. Magnification $\times 40$.

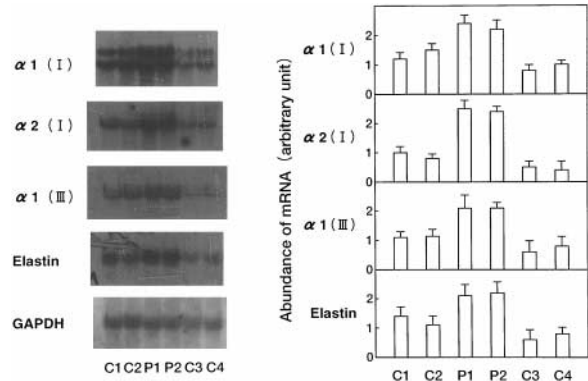


Fig. 2. Levels of matrix protein mRNAs in cultured fibroblasts. RNA was isolated from the fibroblasts of control 1 (lane 1), control 2 (lane 2), patient 1 (lane 3), patient 2 (lane 4), control 3 (lane 5) and control 4 (lane 6). RNA was fractionated on agarose gel and blotted onto nitrocellulose membranes, then hybridized with the probes indicated. The blots were autoradiographed (a). The autoradiograms were scanned with a densitometer. The amount of mRNA relative to GAPDH mRNA was calculated. Values are mean \pm range from two sets of experiments (b).

rich in immature elastic fibres and diminishes with their maturation (16). The elastic fibres accumulating in the lesion may be mature fibres containing a low amount of microfibrils.

The elevation of collagen (hydroxyproline) content in the patients' skin was less than that of elastin (isodesmosine) content (1.4-fold vs 2.0-fold). This may be because basal collagen content of skin is much higher ($\sim 72\%$ of dry skin) than the elastin (1–2% of dry skin) (17). Northern blot assays demonstrated that the expression of type I and type III collagen and of elastin in the patient fibroblasts were elevated. The combined results suggest that the skin lesion may be caused by the accumulation of both collagen and elastic fibres. We therefore now prefer to designate this disease "late-onset focal dermal fibrosis" rather than "late-onset focal dermal elastosis".

Although our results suggest that accumulation of elastic fibres in the patient may be due to the elevated elastin production, excretion of elastin degradation products was normal in the patients. This may be because the elastin content of skin is lower than that of other tissues (17) and skin manifestations of the patients are disseminated papules localizing in specific regions of the body. The mechanism of enhanced elastin expression in this disease is not yet known. Since elastin expression in skin fibroblasts has been shown to be stimulated by sev-

Table II. Immunoreactive elastin peptide concentration in urine

Case	Age/sex	Elastin peptide/ creatinine ($\mu\text{g}/\text{mg}$) (from triplicate experiments)
Patient 1	85/M	3.49
Patient 2	65/F	4.75
Control 1	82/F	2.89
Control 2	76/F	3.78
Control 3	61/F	5.32
Control 4	36/F	4.17
Control 5	40/M	6.06

eral factors including IL-1 β (18), IL-10 (19) and TGF β (20, 21), these factors may be involved in the pathogenesis of the disease.

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