Elastin cross-linking in the skin from patients with amyotrophic lateral sclerosis

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Abstract
Two cross-links unique to elastin, desmosine and isodesmosine were measured and compared in skin tissue (left upper arm) from 10 patients with amyotrophic lateral sclerosis (ALS) and from seven age-matched controls. The contents of desmosine and isodesmosine were significantly decreased $(p < 0.01$ and $p < 0.01$, respectively) in patients with ALS compared with those of controls, and were negatively and significantly associated with duration of illness in ALS patients $(r = -0.77, p < 0.01$ and $r = -0.65, p < 0.05$, respectively). The decline in skin desmosine and isodesmosine is more rapid in ALS than in normal ageing. Thus cross-linking of skin elastin is affected in ALS.

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Several studies have reported an alteration of skin pathology in amyotrophic lateral sclerosis (ALS). \(^1\)\(^-\)\(^4\) Recently it was found that the collagen content of skin from patients with ALS was significantly lower than normal,\(^1\) and that there was a decrease in the content of nonreducible, stable cross-link, histidinohydroxylysino-norleucine (HHL) and an increase in the content of two reducible iminium cross-links, dehydrohydroxylysino-norleucine (deH-HLNL) and dehydrohistidino-hydroxymerodesmosine (deH-HHMD) with duration of illness in the skin collagen of patients with ALS.\(^4\) However, little is known concerning the biochemical nature of skin elastin in ALS.

Elastic fibres are found in most connective tissue with collagen and polysaccharides, and can stretch to several times their length and then rapidly return to their original size and shape when the tension is released. Elastin is the major component of elastic fibres and therefore ideally suited for this role partly due to a series of lysine-derived, covalent cross-links.\(^2\) These cross-links are formed extracellularly and contribute to the remarkable stability of the elastic fibre. Of these cross-links, desmosine and its isomer, isodesmosine, are unique to elastin in mammalian tissues and thereby, provide a specific marker for this protein.\(^5\) Although it is known that the initial step in elastin cross-linking involves the conversion of peptidyl lysine to 5-aminomethyl-5-carboxypentanal by the action of lysyl oxidase,\(^6\) the detailed mechanism of formation of desmosine and isodesmosine is still unknown. Since the same initial step is required in the formation of collagen cross-links which we have found to be disrupted in skin tissue in ALS,\(^4\) we have now quantified cross-links of elastin in skin at various stages of ALS and compared the results to those of controls.

Patients and methods
PATIENTS
There were 10 patients with ALS (mean age, 59 years; range, 52–66 years) and seven age-matched controls with other neurological or muscular diseases (mean age, 61 years; range, 55–71 years). All patients with ALS had the presence of both upper and lower motor neuron signs, clear evidence of progression, normal nerve conduction velocities and late responses, and electromyographic evidence of diffuse denervation. Evaluation included a detailed history and physical examination, and extensive haematological, biochemical, electrophysiological, and radiological testing. At least three neurologists agreed on the diagnosis in each case. The diagnoses in the control group were spinocerebellar degeneration, polymyositis, Alzheimer’s disease, Parkinson’s disease, cerebral infarction, progressive muscular atrophy, and myasthenia gravis in one patient each. Informed consent was obtained from all patients with ALS and controls. Punch biopsy specimens of skin overlying the left biceps were taken following local anaesthesia with 1% procaine hydrochloride, and were stored at $-80^\circ$C until use.

PREPARATION OF SAMPLES
The skin was carefully trimmed of hair and subcutaneous fat. All preparations were carried out at 4°C. Samples were pulsed under liquid nitrogen using a Spex Freezer Mill (Spex, Metuchen, New Jersey) to a fine powder, and then washed with cold 0.015 M N-trishydroxymethyl-2-aminoethanesulfonic acid (TES) buffer, pH 7.4 to remove some of the soluble material and serum proteins. The insoluble fraction was extensively dialysed against cold distilled water, and lyophilised.
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![Graph showing desmosine and isodesmosine levels in ALS patients compared to controls.](image)

**Figure 1** The contents of desmosine (A) and isodesmosine (B) in patients with ALS and control subjects. The contents of desmosine and isodesmosine are significantly decreased in patients with ALS compared with those of control subjects (p < 0.01 and p < 0.01, respectively). Bars show mean (SD); dw = dry weight of the sample.

**Results**

The contents of desmosine and isodesmosine were significantly decreased (p < 0.01 and p < 0.01, respectively) in patients with ALS [mean (SD) 0.94 (0.33) and 0.74 (0.30) nmoles/mg dry weight; range, 0.36–1.51 and 0.29–1.33 nmoles/mg dry weight, respectively] compared with those of controls [mean (SD) 1.43 (0.30) and 1.19 (0.21) nmoles/mg dry weight; range, 1.16–2.03 and 0.92–1.52 nmoles/mg dry weight, respectively] (fig 1). The contents of desmosine and isodesmosine were negatively and significantly associated with the duration of illness in patients with ALS (r = −0.77, p < 0.01 and r = −0.65, p < 0.05, respectively) (fig 2). These data indicated that the amount of mature cross-links of elastin decreased as a function of duration of disease in ALS. The ratio of desmosine and isodesmosine was relatively constant [mean (SD) 1.29 (0.31); range, 1.18–1.41] and there was no significant change in the proportion of these two cross-links in all samples analysed. These ratios were in good agreement with previous reports.10

**Discussion**

It was reported that the amount of desmosine and isodesmosine decreased in elastins isolated from both aortas and pulmonary tissues of humans with increasing age.11 In our study it was demonstrated that the content of desmosine and isodesmosine in ALS patients decreased with duration of illness. The decrease in mature cross-links of skin elastin in ALS patients during illness takes place more rapidly than would be expected in normal ageing.

Fuller et al11 found degenerative elastic tissues and fragmentation of elastic fibres with altered collagen bundles in the skin of ALS patients. Störtebbecker et al,12 who studied biopsied samples of the temporal artery in 12 ALS patients and nine age-matched controls, showed that discontinuities, splitting, and multiple fragments of the internal elastic membrane were observed more frequently in ALS patients than in controls. These changes are considered to reflect normal ageing processes,13 suggesting that morphological changes caused by the ageing process in the elastic component occur more quickly in ALS patients than in controls. The biochemical data presented in this report are consistent with these morphological findings in ALS patients.

The content of desmosine in various elastin preparations has been shown to be fairly constant.14 Consequently, assay of desmosine and isodesmosine, which account for about 1.5 and 1.0 residues per 1000
Amino acids, respectively, can be used as a measure of the amount of elastin in tissues. Based on this assumption, it is likely that there is a marked decrease in the content of elastin in the skin of patients with ALS. Because the immediate precursors of desmosine and isodesmosine are not known, it is not possible at present to ascertain whether these precursors increase as desmosine and isodesmosine decrease, in a manner similar to HHL cross-linking in collagen of ALS skin.

The presumed mechanism of elasticity of tissues is based on the continuity of the network of elastin polypeptides interconnected by stable cross-linkages predominantly in the form of desmosine and isodesmosine. Alterations in the supramolecular organisation of the elastic fibre network could lead to mechanical alterations in the skin, which would manifest as wrinkled, loose, and sagging skin, as noted during cutaneous ageing. In support of this concept are several observations made on patients with either inherited or acquired cutaneous diseases with elastic fibre abnormalities. Cutis laxa is characterised by decreased elasticity of skin due to a decrease in skin elastin detected by reduced desmosine content, and stretching of the skin of this disease produces delayed recoil. The skin of ALS patients in its late stage loses elasticity. When the skin is stretched, it returns very slowly to its original position (“delayed return phenomenon”), similar to that found in cutis laxa. Accordingly, a marked decrease in the amount of elastic fibres in the skin of ALS could well explain this specific clinical finding of ALS patients.

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