Epidermal Transglutaminase in the Ichthyoses

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Membrane-bound transglutaminase (TGm) is responsible for the cross-linking of proteins to form the cornified envelope. Since abnormalities have been reported in the envelope in certain ichthyoses, we have carried out a survey of TGm concentrations in scales from these disorders. Surprisingly, a striking and specific increase in enzyme activity was found in patients with non-erythropoietic autosomal recessive lamellar ichthyosis. It is not clear how this increase is related to the underlying recessive mutation.

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Terminal differentiation (“programmed cell death”) takes place around the granular layer and is responsible for the formation of the stratum corneum (1). A central part of this process is the cross-linking of cellular proteins (such as involucrin) to form the cornified envelope (2–6). The formation of cross-links is catalysed by the membrane-bound enzyme transglutaminase (TGm), which forms Nγ-glutamyl-ε-lysine isopeptide bonds.

It has recently been reported that in certain genetically determined disorders of keratinization there are marked morphological and biochemical abnormalities in the cornified envelope (7, 8), and it is possible that these abnormalities may reflect abnormalities of TGm. Since histochemical observations have shown that TGm persists in stratum corneum (9–12), we have used scales as a convenient material for determination of this enzyme. The transglutaminase activity was measured in a range of monogenic disorders of keratinization, and compared with values determined in psoriasis and in normal stratum corneum.

METHODS AND MATERIALS

Patients

All patients had been examined either at the Department of Dermatology, University Hospital, Nijmegen (NL) or the Department of Dermatology, Münster (FRG). The study included material from patients with the following ichthyoses: erythrodermic autosomal recessive lamellar ichthyosis (EARLI, n=5), non-erythropoietic autosomal recessive lamellar ichthyosis (NEARLI, n=2), Netherton syndrome (n=2), X-linked recessive ichthyosis (XRI, n=5), autosomal dominant ichthyosis vulgaris (ADIV, n=5), X-linked dominant ichthyosis (XDI, n=3), bullous ichthyosiform erythroderma (BIE, n=5). Two additional specimens were obtained from NEARLI patients during retinoid therapy, one being from a patient previously sampled in the untreated group. For comparison, scales obtained from psoriatics (n=6) and from healthy individuals (n=6) were assayed. In the large and heterogeneous ichthyosis group, all patients fulfilled the clinical criteria that are typical of the various disorders involved (13).

The patients (22 M, 15 F; age range 1–74) had not used any medication for at least 3 months before the scales were sampled, unless specifically indicated below. Scales were collected from various regions of the body, with the exception of the palms and the soles. Normal stratum corneum was taken from the back and the lower legs of healthy volunteers (4 M, 2 F; age range 24–57) by gently scraping with a scalpel blade. Scales were stored dry at −20°C prior to measurement of TGm.

Transglutaminase assay

The scales were weighed (about 2–4 mg) before homogenizing (all-glass Potter-type grinder, filled with an ice-jacket) in 1 ml buffer (50 mM Tris, 10 mM CaCl₂, 1 mM EDTA, 2 mM freshly prepared dithiothreitol; pH 8.1). After centrifugation (10 min, 15000 g), the pellet was resuspended in 0.5 ml buffer and sonicated for about 5 s (“particulate fraction”). TGm activity was assayed by measuring the binding of [14C]-labelled putrescine to α-casein. Aliquots of 10 μl Triton X-100 (5%) and 20 μl α-casein (25 mg/ml) were added to 50 μl “particulate fraction”. After adding 20 μl of a solution containing 6 nmol putrescine and 0.1 μg [14C]-putrescine the mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 1 ml ice-cold trichloroacetic acid (TCA) (5%) containing 1 mM putrescine and centrifuged (5 min, 3000 g). The pellet was redissolved in 100 μl NaOH (0.5 M) and precipitated again with 1 ml TCA solution. The final pellet was taken up in 0.5 ml NaOH and 0°C was measured with a scintillation counter (isoscip-300).

All assays were performed in duplicate. After subtraction of the appropriate blanks, TGm activity was calculated as pmol [14C]-incorporated into TCA-precipitable protein per min.

Statistical analysis

Analysis of variance was carried out for diagnosis using Duncan’s multiple range test with a SAS package and a VAX computer system.

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DISCUSSION

Our present data confirm earlier observations that chemical (8, 14) or enzymatic (15) analysis of scales may provide a simple, non-invasive approach for obtaining information of diagnostic value or pathogenetic significance in this group of disorders.

The TGₙ concentrations seen in the non-erythrodermic form of lamellar ichthyosis (NEARLI) are clearly increased and appear to be unique for this disorder. This finding stands in sharp distinction to the normal values seen in the erythrodermic form, and provides further support for the division of the lamellar ichthyoses into these two subgroups (15, 16). The simplicity and reproducibility of the assay makes it a hopeful candidate for a practical diagnostic test. The pathogenetic significance is so far uncertain, since it is by no means clear how the overproduction of this enzyme could be linked to the underlying recessive mutation.

Reduction of TGₙ activity in NEARLI following retinoid therapy is of considerable interest since it has been established that retinoids block the transcription of this enzyme, at both the RNA (17) and protein levels (18–20), and since retinoids are remarkably effective in the treatment of this disease (13). Longitudinal studies on TGₙ levels in patients receiving retinoid therapy are currently in progress.

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Topical Meclocycline Sulfosalicylate, Benzoyl Peroxide, and a Combination of the Two in the Treatment of Acne Vulgaris

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One hundred and six patients with acne vulgaris of the face were treated for 10 weeks with either topical meclencycline sulfosalicylate, topical benzoyl peroxide or both preparations. A randomized, double-blind parallel group study was used. Benzoyl peroxide proved more effective than meclencycline in reducing acne lesion counts, while local side effects were more common in the benzoyl peroxide-treated patients. The combined treatment was of intermediate efficacy with fewer local side effects.

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Combined treatment with topical clindamycin and benzoyl peroxide and with topical erythromycin and benzoyl peroxide have shown advantages over treatment with either of the three agents alone (1, 2). Meclocycline sulfosalicylate is a tetracycline derivative developed for the topical treatment of acne vulgaris. In this study the three topical treatments of acne vulgaris: 1) meclencycline sulfosalicylate, 2) benzoyl peroxide and 3) the combination of the two were compared.