Epidermal Transglutaminase in the Ichthyoses

CANDIDA A. E. M. VAN HOOIJDONK,[1] PETER M. STEIJLEN,[1] MIEKE BERGERS,[1] PAUL D. MIER, [1] HEIKO TRAUPE[2] and RUDOLF HAPPLE[1]

[1]*Department of Dermatology and* [2]*Department of Human Genetics, University Hospital, Nijmegen, The Netherlands*

Membrane-bound transglutaminase (TG_m) 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 TG_m concentrations in scales from these disorders. Surprisingly, a striking and specific increase in enzyme activity was found in patients with non-erythrodermic autosomal recessive lamellar ichthyosis. It is not clear how this increase is related to the underlying recessive mutation.

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C. A. E. M. van Hooijdonk, Department of Dermatology, University Hospital, Ph. van Leydenlaan 25, 6525 EX Nijmegen, The Netherlands.

Terminal differentation ("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 (TG_m), which forms γ-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 TG_m. Since histochemical observations have shown that TG_m 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-erythrodermic autosomal recessive lamellar ichthyosis (NEARLI, n=5), Netherton syndrome (n=2), X-linked recessive ichthyosis (XRI, n=5), autosomal dominant ichthyosis vulgaris (ADIV, n=5), X-linked dominant chondrodysplasia punctata (CPXD, 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 TG_m.

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 $CaCl_2$, 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").

TG_m activity was assayed by measuring the binding of ^{14}C-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 µCi [^{14}C]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 ^{14}C was measured with a scintillation counter (Isocap-300).

All assays were performed in duplicate. After subtraction of the appropriate blanks, TG_m activity was calculated as pmol ^{14}C 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.

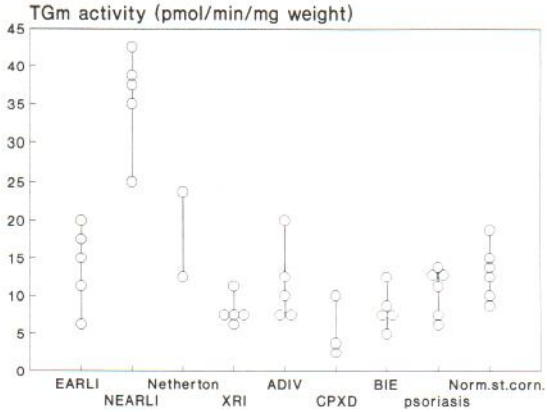

Fig. 1. Transglutaminase activity in normal stratum corneum and scales of individual patients with various disorders of keratinization. TG_m activity is expressed as pmol/min/mg weight.

RESULTS

Preliminary experiments indicated that the enzyme assay was linear for about 45 min. The use of a low concentration of a non-ionic detergent (0.5% final concentration Triton X-100) gave a slight increase in TG_m activity (14%), but higher concentrations had no further effect. The assay was exceedingly sensitive, experimental values being at least 10-fold above the blank. Reproducibility was good, duplicates averaging within 1.7% of the mean. The TG_m activity of stratum corneum obtained from healthy volunteers was similar in trunk and lower leg ($p>0.05$). The mean value of the two specimens was therefore used to calculate the normal range (Fig. 1). The enzyme appeared to be remarkably stable; no loss was observed at −20°C, and less than 30% was lost during 8 weeks' storage at room temperature.

Fig. 1 shows the TG_m levels of individual subjects expressed as pmol per min per mg dry weight. There is a striking increase in enzyme activity in all NEARLI patients, the mean value showing about a 3-fold elevation over the controls ($p<0.05$). The TG_m concentrations of the other patient groups were relatively normal ($p>0.05$ in all cases).

TG_m activity was measured in two additional specimens from patients diagnosed as NEARLI who were currently being treated with acitretin. Although the TG_m activities, 24 and 27 pmol per min per mg weight, respectively, were still elevated, these values indicate some reduction when compared with untreated patients.

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 TG_m 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 TG_m 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 TG_m levels in patients receiving retinoid therapy are currently in progress.

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