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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Pachyonychia Congenita

Electron Microscopic and Epidermal Glycoprotein Assessment Before and During Isotretinoin Treatment

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- Two patients, a father and son, with pachyonychia congenita were treated with orally administered isotretinoin because the extreme deformity and discomfort associated with their massive keratoderma interfered with their work and school, respectively. While clinical benefits could not be sustained, electron microscopic findings compatible with suppression of abnormal keratinization were observed. In addition, skin biopsy samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the gels were then subjected to a lectin overlay technique with concanavalin A labeled with iodine 125. The distribution of specific glycoproteins was found to be different for lesional as against normal epidermis. The procedure was repeated after oral treatment with isotretinoin. The labeled glycoprotein pattern of the lesional epidermis was clearly distinguishable from both the pretreatment lesional and the normal epidermis; it was mostly intermediate between the two. The normal epidermis was virtually unaffected by the retinoid treatment.

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Pachyonychia congenita is a rare genodermatosis in which there is severe and disabling hyperkeratosis of the palms and soles, subungual hyperkeratosis with marked thickening of the distal portions of the nails, and oral leukokeratosis. Other reported possible manifestations include the following: hair abnormalities, hyperhidrosis, follicular hyperkeratosis, especially of the elbows and knees, cornal changes, natal teeth, and epidermal inclusion cysts. Unlike dyskeratosis congenita, the oral lesions do not have a notable tendency toward malignant degeneration. The syndrome is transmitted by an autosomal dominant gene with high penetrance and variable expression. Electron microscopic studies have shown an increase in the number and thickness of tonofibrils as well as abnormal keratohyalin granules and intracellular and intercellular edema.

Dermatoses characterized by abnormal keratinization that have been successfully treated with oral doses of isotretinoin include the following: psoriasis, the ichthyoses, Darier's disease, pityriasis rubra pilaris, mal de Meleda, and some other palmoplantar keratodermas. Isotretinoin therapy for keratinizing disorders has been associated with following morphologic effects: an increase in the intercellular gap junctions in the epidermis and a decrease in the number of keratohyalin granules, tonofibrils, and desmosomes. Retinoids also influence cell division, RNA synthesis, protein synthesis, posttranslational glycosylation of proteins, prostaglandin biosynthesis, lysosomal membrane stability, and cell membranes. In summary, evidence exists that the retinoids may suppress, but not reverse, the manifestations of disorders of keratinization. Animal studies suggest that changes in transepidermal water loss and a loss of epidermal cohesion may account for clinical "keratolytic" effects of retinoids in disorders of keratinization.

Brysk and Snider have demonstrated changes in the glycoproteins of epidermal cells at different stages of differentiation using a variety of tracer techniques, including lactoperoxidase iodination and reaction with several lectins. Brysk et al. have also demonstrated that concanavalin A can distinguish among diseases of altered epidermal differentiation. Retinoids stimulate the biosynthesis of mannose-rich glycoproteins. Concanavalin A has a specific affinity for mannose-containing sugars. We, therefore, used this lectin as a probe of glycoprotein changes associated with retinoid treatment of pachyonychia congenita.

We describe a father and son with severe disabling pachyonychia congenita. Experimental therapy with oral isotretinoin was instituted in dosages from 2 mg/kg/day to 3 mg/kg/day, and electron microscopic and biochemical assessments were performed on lesional and normal skin before and during experimental therapy.

SUBJECTS, MATERIALS, AND METHODS

Patients

A 39-year-old man and his 6-year-old son were initially observed at the Dermatology Clinic at the University of Texas Medical Branch, Galveston, in March 1981, with thickened nails and severe keratoderma of the palms and soles. Abnormal nails were noted at birth both in the father and the son. The keratotic lesions on the palms and soles developed early in childhood in both patients (Figs 1 and 2). In addition, both father and son had keratotic patches on the extensor aspects of the knees. The father...
Light Microscopy

Shave biopsy specimens of hyperkeratotic plaques from the knees were obtained from each patient at baseline. Specimens were fixed in formaldehyde solution and stained with hematoxylin-eosin.

Electron Microscopy

Punch biopsy specimens (4 mm) were taken from involved skin from the knee of each patient at baseline and from adjacent sites eight weeks after initiation of therapy. Tissues were fixed in 2% glutaraldehyde and 1% osmium tetroxide, block stained in uranyl acetate, dehydrated through alcohol, and embedded in Spurr resin. Thick sections (1 μm) were stained with toluidine blue for orientation. Thin sections were stained with lead citrate and examined with a transmission electron microscope.

Biochemical Studies

Before and after retinoid treatment, superficial shave biopsy specimens were taken of both lesional and normal skin. To provide additional controls, a skin sample from an unrelated patient was heated separated at 60 °C for 30 s into dermis and epidermis. Tissue samples were mixed at 4 °C in Dulbecco’s phosphate buffered saline, pH 7.2, containing 2mM phenylmethylsulfonylfluoride, homogenized in a glass-grass tissue grinder, then sonicated for two minutes in an ultrasonic cell disruptor. The dehydration and gel overlay technique with concanavalin A has been previously published. Briefly, the cells were solubilized by boiling in sodium dodecyl sulfate (SDS) buffer, and the resulting total cell extracts were loaded onto the SDS-polyacrylamide gel electrophoresis (PAGE) gels, with an equal amount of protein present in each lane of the gel. After electrophoresis, the gels were overlaid with concanavalin A labeled with iodine 125 with or without 4% of the competing sugar, α-methylmannoside.

RESULTS

Clinical

The keratotic papules on the knees of both patients cleared within one month after starting oral isotretinoin therapy. The leukekeratotic plaques on the oral mucosa of the father also cleared. However, there was no objective clinical improvement of the keratoderma on the palms and soles of either patient. No blistering was observed at these sites. The nails were not affected by treatment. In both patients chelitis, xerosis, and dryness of the oral mucosa developed. Therapy with isotretinoin was discontinued in the father after eight weeks (3 mg/kg/day) due to an elevated serum triglyceride level of 1,500 mg/dL (baseline for father, 202 mg/dL). The son underwent a full 16-week course (2 mg/kg/day). Triglyceride levels were obtained after a 36-hour fast from alcohol and a 14-hour fast that included avoidance of caffeine. The father was seen by an endocrinologist who recommended weight reduction. The patient was non-compliant. A second attempt at isotretinoin therapy (1 mg/kg/day) was discontinued after eight weeks (triglyceride level of 600 mg/dL). Roentgenograms of the son's long bones after treatment showed no premature closure of the epiphyses. All laboratory measurements, except for the serum triglyceride level of the father, were within the normal limits before and during therapy with oral isotretinoin.

Fig. 1.—Pretreatment clinical photograph of father’s hand. Note massive hyperkeratosis and thickened nails.

Fig. 2.—Pretreatment clinical photograph of son’s nails. Note typical thickening.

also had leukokeratotic plaques on the oral mucosa since puberty. Neither patient had corneal changes on slit-lamp examination. There was no evidence of the following: hair abnormalities clinically or on low-power light microscopy, dental abnormalities, or epidermal inclusion cysts. There was no history or evidence of hyperhidrosis or skin blistering. The father had been unable to work for three months due to painful palmoplantar keratoderma that left him confined to a wheelchair. A decision to begin treating both patients with oral isotretinoin (2 mg/kg/day increased to 3 mg/kg/day after one week in the father only) was based on theoretical considerations and on a report of two patients with a similar keratoderma whose conditions improved substantially with this medication. (An investigator's investigational new drug [IND] application was obtained through the permission of Hoffman-LaRoche, Inc, which holds the original IND.) Informed consent was obtained from the patients before beginning therapy.

The following baseline laboratory studies were performed on the father and son with pachyonychia congenita: chest roentgenogram; ECG; urinalysis; fasting cholesterol and triglyceride; complete blood cell count; differential cell and platelet counts; 12-panel blood chemistry; prothrombin time; partial thromboplastin time; Westergren ESR; roentgenogram for bone age determination (child only); sperm count (father only); and skin biopsy. Lipid studies, blood cell counts, and liver function tests were repeated at four-week intervals. Sperm counts and bone age were determined at eight-week intervals.
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Fig 3.—Pretreatment skin biopsy specimen from father’s knee showing hyperkeratosis and acanthosis (hematoxylin-eosin, original magnification ×200).

Fig 4.—Pretreatment electron micrograph from father’s knee. Note stratum spinosum cells with large tonofilbr aggregates and prominent intracellular and intercellular edema (original magnification ×13,500).

Light Microscopy

Routine histologic specimens were taken from each patient at baseline. These disclosed marked hyperkeratosis, acanthosis, and occasional keratotic plugs in sweat ducts (Fig 3).

Electron Microscopy

Pretreatment.—The basal cells showed increased tonofilbrils that were more dense than normal. This

Fig 5.—Posttreatment electron micrograph from father’s knee. Note more normal tonofilbr bundles and intracellular and intercellular edema (original magnification ×13,500).

ultrastructural finding became more pronounced, and large masses of tonofilbrs were found inside some cells, in the stratum spinosum (Fig 4). Intracellular and intracellular edema also became apparent in the stratum spinosum. Intracellular edema was also noted in the granular layer, along with abundant large keratohyalin granules and large aggregations of dense tonofilbrils. No intercellular edema was noted at this level. The desmosomal attachments remained remarkably dense throughout the epidermal maturation process.

Posttreatment.—The main ultrastructural findings after isotretinoin therapy were related to a slight increase of intercellular and intracellular edema at all levels of the epidermis and a decreased density of tonofilbr bundles. No dense masses of tonofilbrs were found in any of the posttreatment biopsy specimens (Fig 5).

Biochemical Studies

Epidermal glycoproteins that bind concanavalin A are labeled by a lectin overlay technique in which the macromolecules are first separated on SDS-PAGE and then reacted with concanavalin A 125I. Reaction with concanavalin A specifically labels D-glucosyl and D-mannosyl sugar residues. In a control experiment, inclusion of the concanavalin A inhibitory sugar α-methyl-D-mannoside together with the iodinated lectin completely obliterated the uptake of radioactivity from concanavalin A 125I. Figure 6 depicts densitometer scans of autoradiograms obtained with this technique. The scans on the left (a, e, f, and g) are from biopsy specimens of the father with pachyonychia congenita. The top three on the right (b, d, and f) are from the son. Figure 6, h, consists of control scans from an unrelated subject.

As Figure 6, h, shows, the labeling pattern for the dermis differs dramatically from that for the epider-
For the normal epidermis (e, f, solid curve h), there are two major labeled bands, near 40 and 80 Kd. Between them are two less intense peaks near 45 and 50 Kd, with the 45-Kd peak clearly the greater. There is also a band of comparable magnitude near 100 Kd.

Comparing the untreated lesional epidermis (a and b) with the normal, the radioactivity is found at the same molecular weights with some variations in relative intensities. The 40-Kd band appears unaffected; the 80-Kd band is substantially less intense. The 45- and 100-Kd peaks just about disappear, whereas the 50-Kd peak is much more prominent.

The labeling pattern of the retinoid-treated lesional epidermis (c and d) is mostly intermediate between those of the untreated lesional and the normal epidermis. The 50-Kd band is closer to the normal. The 50-Kd peak is enhanced for the treated as against the untreated lesional epidermis (while it is much lower for the normal). Similarly, a less intense peak is evident at 60 Kd, which is nearly indiscernible for either normal or untreated lesional epidermis. The retinoid-treated normal epidermis (g) is essentially indistinguishable from the untreated normal epidermis, except that the 50-Kd peak becomes comparable to the 45-Kd peak.

**COMMENT**

Pachyonychia congenita is a rare keratodermia transmitted by an autosomal dominant gene in which there is considerable hyperkeratosis of the palms, soles, and nails. Clinically, there are three categories of pachyonychia congenita: type 1, symmetrical hyperkeratosis of the palms and soles with follicular keratosis of the body; type 2, similar to type 1 with the addition of leukokeratosis (the most common of the three types); and type 3, similar to type 1 with the addition of corneal changes. Other reported manifestations include hair abnormalities, hyperhidrosis, natal teeth, epidermal inclusion cysts, and white exophytic lesions on the larynx.

Routine histopathologic examination of involved skin in our patients disclosed marked hyperkeratosis and acanthosis with keratinic plugs in the sweat ducts, similar to the findings of Thormann and Kobayasi. There was no evidence of cornoid lamella, which has been reported by Wilkin.

Electron microscopic studies of involved skin in our patients before treatment with isotretinoin showed an increased number of desmosomes throughout the epidermis, increased number and thickness of tonofibrils, intracellular and intercellular edema, and large, abnormal keratohyalin granules. The results were again similar to those of Thormann and Kobayasi and were consistent with a defect in keratinization. Posttreatment electron microscopy studies from areas adjacent to the baseline biopsy specimens disclosed increased intercellular and intracellular edema, and decreased density of tonofibril bundles. These results are compatible with a suppression of abnormal keratinization.

The distribution of specific glycoproteins, as assessed by biopsy, is abnormal.
assessed using the lectin overlay technique on gels obtained after SDS-PAGE electrophoresis from skin biopsy samples, was found to be different for lesional as against normal epidermis. While normal epidermis was virtually unaffected by the retinoid treatment, the labeled glycoprotein pattern of the lesional epidermis was clearly distinguishable from both the pretreatment lesional and the normal epidermis. In summary, while pachyonychia congenita has been described and characterized ultrastructurally, nothing is known of the biochemical manifestations of this disease. Chemical extractions would require several orders of magnitude of more material than is available from biopsy samples such as we used. Nevertheless, we were able to demonstrate differences between the glycoproteins of normal and lesional epidermis, and even modifications associated with retinoid treatment.

While pachyonychia congenita may, at first glance, appear to be primarily a cosmetic disorder, the physical disability from massive keratodermia can take a substantial toll, as illustrated by the father in this family. Topical keratolytic agents (eg, salicylic acid, lactic acid, urea), surgical nail removal, protective footwear, and even hypnosis, x-ray therapy, and high doses of vitamins A and E have been used. While vitamin A has some rational basis for use in keratinizing disorders and is reported to be of some benefit in pachyonychia congenita, its long-term use is contraindicated by its toxicity. None of these treatments, therefore, has produced permanent solution to the notable morbidity caused primarily by the painful hyperkeratosis and fissuring of the palms and soles.

Therapy with isotretinoin was initiated in these patients on sound grounds given current knowledge of the dyskeratosis involved in pachyonychia congenita, the extreme disability experienced by the patients, and the ability of this agent to suppress abnormal keratinization in other similar disease states. Unfortunately, one of our patients had great elevation of serum triglyceride levels, requiring discontinuation of isotretinoin therapy after only eight weeks of treatment. His son was treated for 16 weeks with isotretinoin without any noticeable clinical improvement in the keratodermia on his palms and soles. However, the keratotic patches on the knees of both patients and the leukokeratotic lesions in the mouth of the father cleared after one month of treatment with isotretinoin.

An attempt was made to alleviate the morbidity caused by the disease in these two patients by using oral isotretinoin therapy. However, the clinical results were disappointing in the son, and the father could not continue therapy due to elevated triglyceride levels. Electron microscopic and biochemical studies, however, support an effect by isotretinoin. Future attempts at treatment with isotretinoin should be undertaken with the understanding that currently long-term side effects from continuous therapy with this agent, particularly bone and lipid effects, are unknown. As the aromatic retinoid, etretinate, becomes available in the United States, it is worthy of a clinical trial in severely disabled patients with pachyonychia congenita.

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