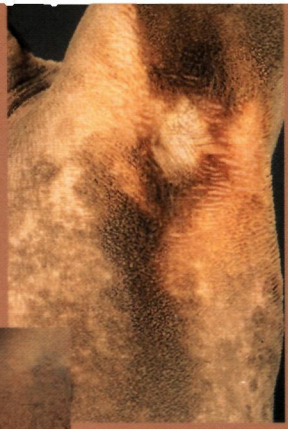
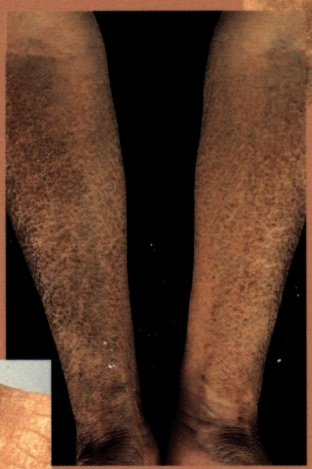
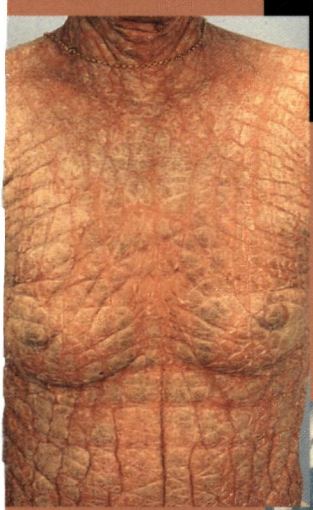
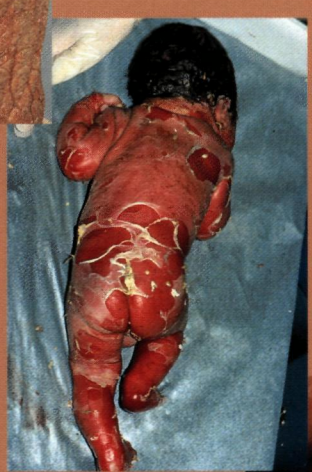


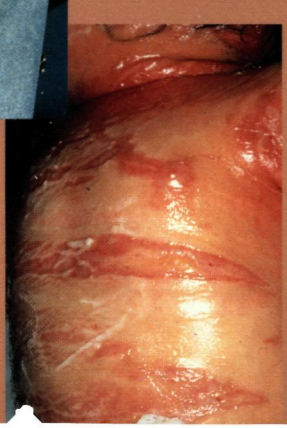


Ichthyosis, Darier's disease and Palmoplantar Keratoderma



New insights in classification and therapy





Georges P.H. Lucker

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Co-promotor	:	Dr. P.M. Steijlen
Manuscriptcommissie	:	Prof.dr. U. van Haelst Prof.dr. J. Schalken Prof.dr. A. Smals

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CONTENTS

General Introduction

Classification and treatment

Disorders of keratinization comprise a large and heterogeneous group of skin diseases, which can be subdivided into hereditary and acquired forms. The majority of hereditary disorders of keratinization are monogenic with a mendelian inheritance. Classification can be made on clinical grounds, histological and biochemical characteristics and by molecular genetic analyses. Clinical description of skin manifestations has to be detailed. History and general examination will reveal whether a defined disorder of keratinization can be regarded as "isolated entity" or alternatively whether the disorder is part of a "syndrome". Histological examination of skin biopsies will reveal whether the abnormality of keratinization can be designated as orthokeratotic, parakeratotic or can be identified as acantholytic or epidermolytic hyperkeratosis. Accompanying inflammation in dermis or epidermis may provide an additional key to the diagnosis of a disorder of keratinization. More recently, molecular genetics has been developed to the extent that some disorders of keratinization, now can be diagnosed at the gene level. Using above mentioned distinct modes of classification, many new disorders of keratinization have been described in recent years. On the other hand, forms which were previously considered to be distinct entities now have been shown to be variants of the same disorder. In the near future, the diagnosis of disorders of keratinization will be confirmed by modern molecular genetics.

The treatment of monogenic disorders of keratinization has not received the broad attention which has been dedicated to frequently occurring polygenic disorders of keratinization such as psoriasis and acne. Although each entity within the group of monogenic disorders of keratinization has a relatively low occurrence in itself, the total group is large. So large, that the general dermatologist is often faced with the need to provide his patient suffering from a monogenic disorder of keratinization the most optimal treatment. An important "point de départ" is the adequate diagnosis. Based on the diagnosis, the natural course of the disease, the individual impairment of quality of life of a patient and the risk/benefit ratio of the available treatments, the dermatologist will select a treatment strategy. Either a long-term treatment or crisis intervention may be undertaken. In many disorders of keratinization systemic retinoids are effective. As serious side effects may be associated with systemic retinoid treatment, the use of this drug is limited. Systemic retinoids are indicated exclusively in those patients who are retinoid responders and in those patients with a very severe involvement. The optimum retinoid dosage is disease-dependent. For reason of side effects of systemic retinoids, development of new therapeutic approaches is of major importance.

This thesis will be focused on three monogenic disorders of keratinization: ichthyosis, Darier's disease and palmoplantar keratoderma. Recently, major advances have been

made in the understanding of ichthyosis and Darier's disease, whereas progress on insight of various representants of palmoplantar keratoderma is more modest. Whereas classification of ichthyosis has been the objective of modern genodermatology, such an integrated approach has not updated the nosology of palmoplantar keratoderma. Therefore, in this thesis the following **major objectives** were outlined:

(i) **Development of new therapeutic approaches for patients with ichthyosis and Darier's disease with special reference to the disease specificity of therapeutic intervention.**

(ii) **Development of an updated integrated nosology of palmoplantar keratoderma and exploration of new treatments.**

Ichthyosis and Darier's disease

Ichthyosis is a general designation for both acquired and hereditary disorders of cornification, clinically characterized by excessive scaling, covering a substantial part of the body. An updated classification of the hereditary ichthyoses has recently been described by H Traupe, who distinguishes four major groups according to their their clinical and biochemical characteristics: isolated vulgar, associated vulgar, isolated congenital and associated congenital ichthyoses. This classification now is developed further in the "époque" of molecular genetics. In Table I the main groups without associated features (isolated ichthyoses) have been listed. For a detailed account on these various diseases the reader is referred to the monography of Traupe (1) and the thesis of Steijlen (2).

In part I of this thesis new therapeutical approaches for disorders of keratinization are presented. We have restricted these studies to the isolated vulgar ichthyoses autosomal dominant ichthyosis vulgaris and x-linked recessive ichthyosis, the isolated congenital ichthyoses bullous ichthyotic erythroderma of Brocq, erythrodermic lamellar ichthyosis and non-erythrodermic lamellar ichthyosis, the associated congenital ichthyoses Sjögren-Larsson syndrome and Comèl-Netherton syndrome and Darier's disease. The reader is introduced briefly into the main features of these disorders. For a more detailed account, the reader is referred to the monography of Traupe (1), various textbooks (3,4) and reviews (5).

Figure 1 illustrates clinical appearance. Histological features are expressed in Figure 2.

Table I. Isolated ichthyoses (adapted from Traupe et al.)

	inheritance	no associated features	gene defect
ichthyosis, vulgar	AD	- autosomal dominant ichthyosis vulgaris	
	X-linked	- x-linked recessive ichthyosis	- steroid sulfatase (6)
ichthyosis, congenital	AD	- autosomal dominant lamellar ichthyosis	
		- bullous ichthyotic erythroderma of Brocq	- keratin 1 or keratin 10 (7)
		- ichthyosis bullosa of Siemens	- keratin 2e (8-10)
		- ichthyosis hystrix of Curth and Macklin	
	AR		- transglutaminase (11)
		- erythrodermic lamellar ichthyosis	- transglutaminase (11)
		- non-erythrodermic lamellar ichthyosis	
	AR/AD		
		- harlequin fetus	

(i) **Autosomal dominant ichthyosis vulgaris (ADIV)** is the most frequent and mildest subtype with an estimated prevalence of 1%. It usually starts during the first year of life, and is characterized by small white to light gray scales covering mainly the trunk and extensor surfaces of the extremities. The flexures are spared. The palmoplantar skin folds usually are pronounced. Keratosis pilaris may be present on the extensor sides of the extremities. In ADIV filaggrin, a structural protein of the corneocyt causing aggregation of keratin filaments into larger filaments, and its precursor profilaggrin, synthesized in the upper granular cell layer and stored in the keratohyalin granules, are both reduced. The reduction correlates with the severity of the disease. Histologically, these biochemical defects are reflected by reduction of the granular cell layer. The course of ADIV is chronic. Bland emollients usually are sufficient to control the condition.

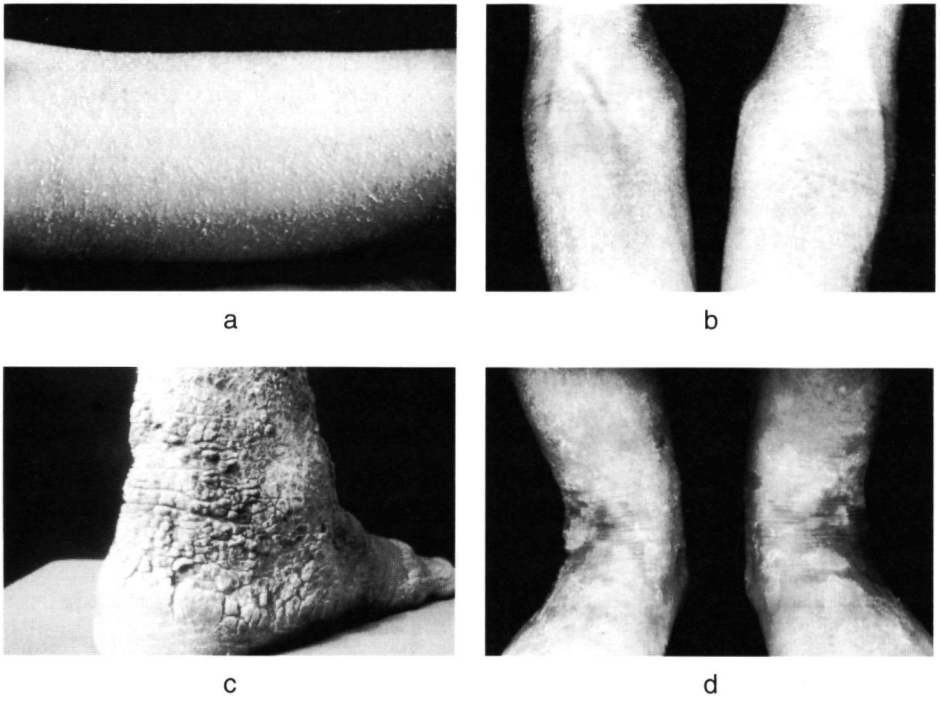
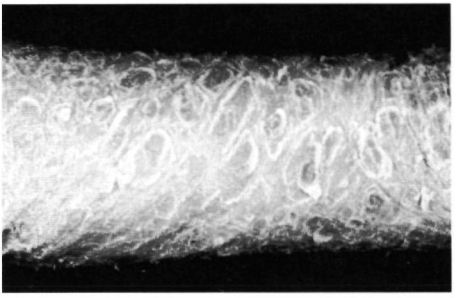


Fig. 1. Clinical appearance of the ichthyoses examined in this this thesis and Darier's disease. a. ADIV. Small white coloured scales on the extensor surface of the upper extremities; **b.** XRI. Small brown coloured scales covering the arms, with exception of the elbow folds; **c.** BCIE. Extensive hyperkeratosis with prominent keratotic ridges; **d.** IBS. Keratotic lichenification of the flexural skin covering the ankle joints with superficially eroded area's, the so-called "mauserung"



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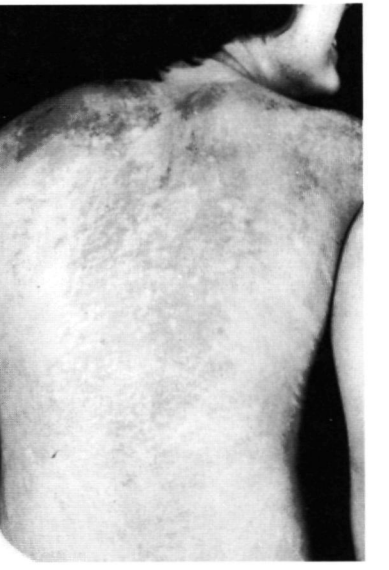

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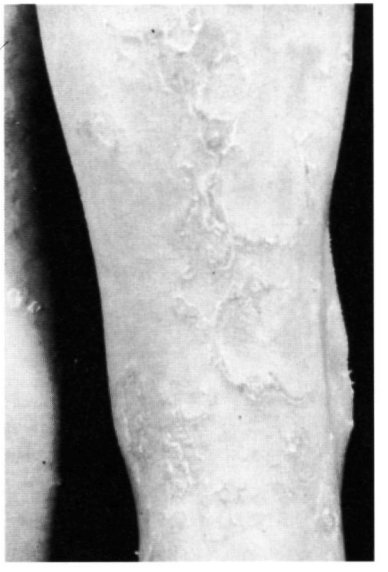

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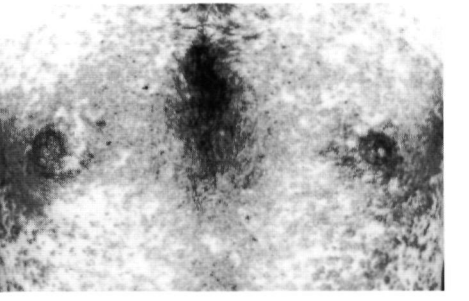


h

Fig. 1. Clinical appearance of the ichthyoses examined in this this thesis and Darier's disease.
e. Lamellar ichthyosis. Prominent large brown coloured scales with some erythema in between: **f.** SLS. Yellow-brown hyperkeratosis with a verrucous aspect. The muscular hypertonus is visualized by the scoliosis; **g.** CNS. Classical ichthyosis linearis circumflexa variant, exhibiting gyrate, serpiginous, double-edged hyperkeratoses. Furthermore, coexisting generalized erythema is visible as observed in the congenital ichthyosiform erythroderma, usually the presenting sign in CNS; **h.** Darier's disease. Red-brownish papules focally confluencing into plaques on the chest.

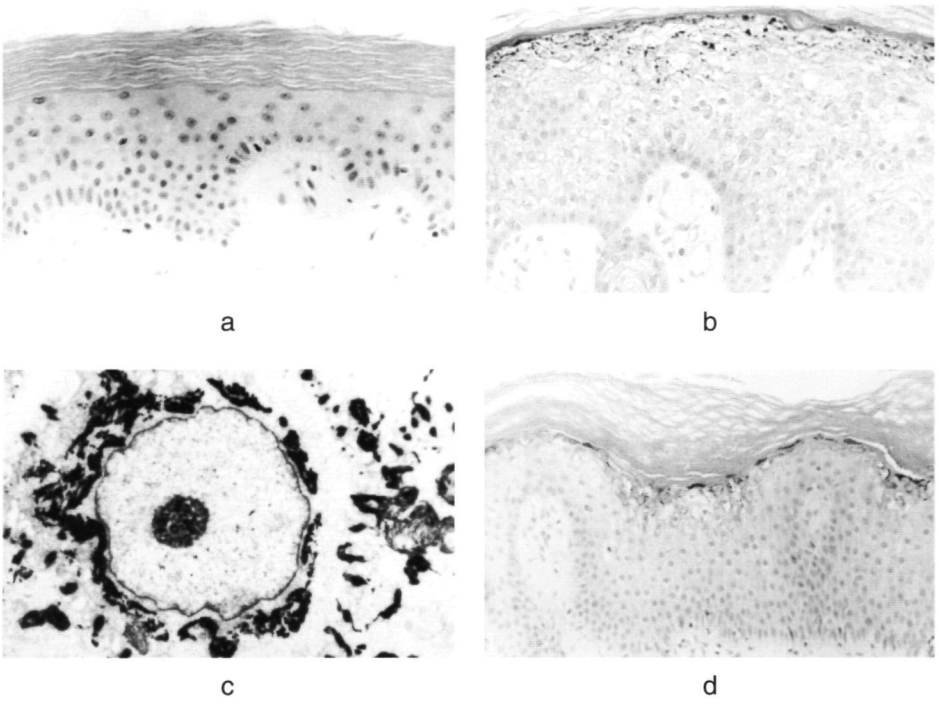


Fig. 2. Histological features of these ichthyoses and Darier's disease. a. ADIV. Mild orthokeratotic hyperkeratosis with absence of the granular cell layer; **b.** BCIE. Epidermolytic hyperkeratosis is expressed in the keratinocytes of the suprabasal compartment which show cytoplasmic edema and perinuclear vacuolization, giving the boundaries between the keratinocytes a blurred appearance, whereas the basal keratinocytes appear normal. Orthokeratotic hyperkeratosis, hypergranulosis and acanthosis reflect the reactive hyperproliferation; **c.** BCIE. Electron micrograph of the spinous cell layer. Aggragated tonofilaments are aranged around the nucleus in a shell-like formation. Clumps of irregular tonofilaments are localized in the cell periphery. These changes result in disruption of the normal tonofilament insertion in the desmosomal plates; **d.** IBS. The same features are observed as in BCIE, though the epidermolytic hyperkeratosis is confined to the upper spinous and granular cell layer. The epidermis shows plump, confluent rete ridges

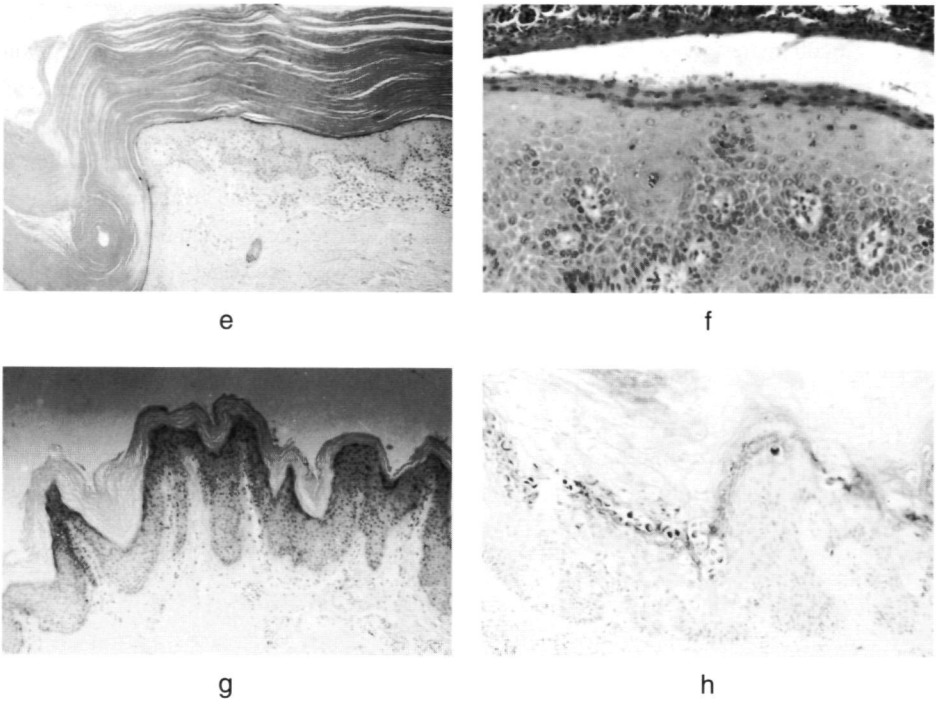


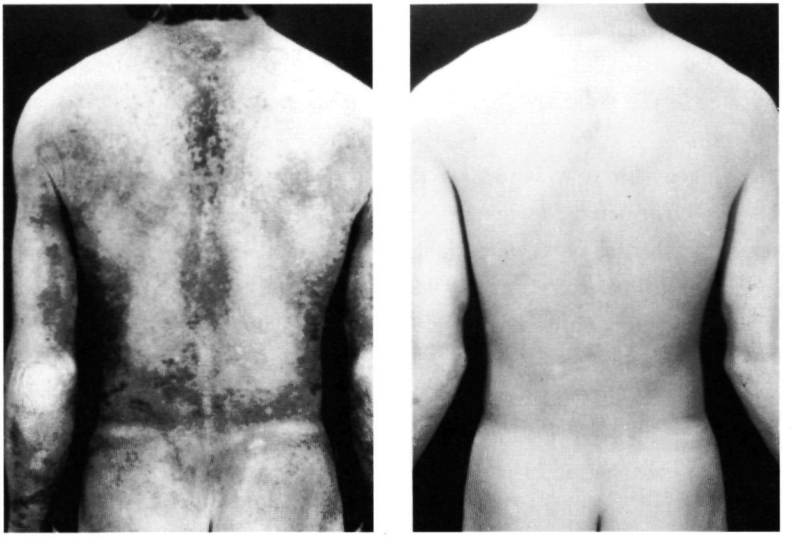
Fig. 2. Histological features of these ichthyoses and Darier's disease.
e. Lamellar ichthyosis. Massive orthohyperkeratosis and papillomatosis of the epidermis. The upper dermis shows a mononuclear perivascular cell infiltrate; **f.** CNS. Parakeratotic cornified cell layer containing masses of pyknotic granulocytes, intracorneal cleft formation, papillomatosis and perivascular inflammatory infiltrates focally invading the epidermis; **g.** SLS. Moderately increased, predominantly orthokeratotic cornified cell layer, acanthosis and some papillomatosis, resembling the features of lamellar ichthyosis; **h.** Darier's disease. Characteristic is the disruption of the cell interconnections with widening of the intercellular space (acantholysis). Massive orthohyperkeratosis, focal parakeratosis, and acanthosis reflect the reactive hyperproliferation.

(ii) **X-linked recessive ichthyosis (XRI)** has an estimated prevalence of 1:2000 - 1:6000 males. XRI has its onset, like ADIV, in the first year of life. Brown-coloured scales are found, covering the extremities. The skin folds of elbows and knees are spared. The trunk may be affected to a variable extent. The underlying defect is a deficiency of the enzyme steroid sulfatase, katalyzing the breakdown of cholesterol sulphate (6). Accumulation of cholesterol sulphate in the cornified cell layer, causes increased cohesion between the corneocytes, and therefore retention hyperkeratosis. Two other features of steroid sulfatase deficiency are cryptorchidism and insufficient cervical dilatation, causing prolonged delivery. The steroid sulfatase locus does not fully escape X inactivation. Consequently, steroid sulfatase activity in most female carriers is below that in normal males. When enzyme activity is rather low, this is visible as a fine scaling especially on the lower legs, occurring in about 25% of female carriers. Although patients may respond sufficiently to topical emollients, many patients require a more effective treatment. Sometimes, systemic retinoids might be indicated.

(iii) **Congenital bullous ichthyotic erythroderma of Brocq (CBIE).** This is a sporadically occurring disease. Immediately after birth a substantial part of the skin is covered with erythema and large blisters. During the first year of life, both erythema and blisters improve gradually. At the same time, hyperkeratosis appears, especially in the skin folds. After removal of these hyperkeratoses, some erythema is visible. Blisters may appear after localized traumata, pressure or friction. The histological hallmark is epidermolytic hyperkeratosis, showing clumping of keratin filaments, present in the entire suprabasal compartment. The underlying genetic defect has been found in the genes coding for keratin 1 or keratin 10 (7). Mutations in keratin 1 cause CBIE with palmoplantar keratoderma, whereas mutations in keratin 10 lead to the phenotype of CBIE without palmoplantar keratoderma. The epidermolytic epidermal nevus probably represents a mosaic of CBIE, due to a somatic mutation of the CBIE gene during embryogenesis. Systemic retinoids are indicated and may cause a dramatic improvement (Figure 3). However, the dosage should not be to high in order to prevent blister formation.

(iv) **Ichthyosis bullosa of Siemens (IBS).** Patients suffer from birth from blistering and dark-gray hyperkeratoses. In contrast to BCIE, erythroderma is absent and hyperkeratoses are more localized. As in BCIE the flexural surfaces of the extremities are involved, while the palmoplantar skin is spared. Hyperkeratoses display a lichenified appearance on the extensor sides of the joints. Characteristic are the superficially eroded areas on the lower aspects of the legs and the backs of hands and feet (mauserung). Blisters may develop after minor mechanical

traumaespecially when combined with profuse sweating. With increasing age, the degree of blister formation and the extent of keratotic involvement tend to improve. IBS is histologically characterized by epidermolytic hyperkeratosis, though less marked as in BCIE and confined to the upper epidermal cell layers. The underlying genetic defect has been found in the keratin 2e gene (8-10). Systemic retinoids are indicated. The maintanace dose is lower than required in BCIE.



a b

Fig. 3. Congenital Bullous Ichthyotic Erythroderma of Brocq: a. Before treatment; **b.** After 10 weeks of treatment with etretinate (50 mg/day).

(v) **Erythrodermic lamellar ichthyosis (ELI)**, again a relatively rare condition, appears immediately after birth. The most outstanding feature is erythroderma. Sometimes, the babies are encased in a thick cornified shell, covering the entire skin surface. This so-called collodion membrane is shed off in the first days after birth and replaced by relatively small translucent, white-greyish scales on an erythematous underground. In a few patients the erythroderma fades and is no longer visible in later life. Most patients express marked palmoplantar keratoderma. Systemic retinoids are indicated in this condition.

(vi) **Non-erythrodermic lamellar ichthyosis (NELI)** has its onset, like ELI, immediately after birth. The disorder has a sporadic occurrence. Large brown-

coloured scales are covering the entire skin. Except for some erythematous stripes between the scales, no erythema is present. Palmoplantar keratoderma is only minimal in contrast to the marked palmoplantar keratoderma in the erythrodermic subtype. NELI patients may also be born as collodion-baby. Recently, mutations of keratinocyte transglutaminase have been established to cause lamellar ichthyosis (11). Disturbed membrane anchorage of transglutaminase could alter loricrin and involucrin cross-linkage and as a result the formation of the cornified envelope. In the literature dealing with the genetic background of lamellar ichthyosis, the clinical distinction between an erythematous and non-erythematous variant is not made, though lamellar ichthyosis is rather regarded as a congenital skin disorder characterized by generalized scaling and variable redness. Systemic retinoids are indicated in this condition, however, the dosage has to be low.

(vii) **The Sjögren-Larsson syndrome (SLS)** is a rare, severe, neurodegenerative skin disorder, characterized by congenital ichthyosis and neurologic manifestations. The disease is inherited as an autosomal recessive trait. Ichthyosis manifests at birth as a generalized fine scaling only, whereas the typical clinical features develop during the first year of life. During childhood a typical yellow- brown verrucous hyperkeratosis appears especially covering the flexures, the lower abdomen and the neck, whereas thin adherent scales may be seen on the legs. The hyperkeratosis is considered hyperproliferative. Neurologic manifestations consist of mental retardation and spastic bi- or tetraparesis and have their onset between 4 and 30 months of age. Ophthalmological examination may reveal small glistening dots in the macular region of the retina, representing fatty degeneration of retinal microglia. Recently, mutations in the fatty aldehyde dehydrogenase (FALDH) gene localised on chromosome 17, have been found in SLS, leading to diminished enzyme activity (12). Although the deficiency of FALDH activity is the cause of SLS, the pathogenetic mechanism responsible for neurologic and cutaneous symptoms is still unclear. Owing to the involvement of FALDH in fatty alcohol oxidation, it is thought that the symptoms arise from accumulation of fatty alcohols, which subsequently are incorporated in lipids in keratinocytes and neurons. This might cause derangement of normal lipid composition of the stratum corneum and nerve sheats, and therefore explain both dermatological and neurological symptoms.

(viii) **The Comèl-Netherton syndrome** is inherited as an autosomal recessive trait and characterized by a distinctive type of ichthyosis in combination with specific hair anomalies, associated with a number of non-dermatological symptoms. Ichthyosis may start with congenital ichthyosiform erythroderma (CIE) which in classical cases evoluates into ichthyosis linearis circumflexa (ILC). ILC is characterized by

erythematous, gyrate, serpiginous patches surrounded by double-edged fine hyperkeratotic margin on the trunk and extremities. CIE, however, may persist in later life, strongly resembling the clinical picture of erythrodermic lamellar ichthyosis. Immune deficiency, leading to infections of the skin and internal organs, is an important non-cutaneous manifestation. According to Traupe, immune defects are more severe in those patients exhibiting the CIE phenotype. Enteropathy, growth retardation, failure to thrive and dehydration are other non-cutaneous manifestations reported especially in the CIE phenotype, whereas these features are absent or mild in the ILC phenotype. Trichorrhexis invaginata is the characteristic hair shaft abnormality, resulting in brittle hair, breaking easily. Histologically, deposition of eosinophilic material in the stratum corneum, a reduced to absent granular cell layer, and subcorneal cleft formation, are the most important epidermal changes, whereas the dermis shows a marked inflammatory infiltrate, invading the epidermis. On electron microscopy, a marked decrease of the tonofilament- desmosome complex, lack of lamellar bodies and keratohyalin granules are observed.

(ix) **Darier's disease** (13) is inherited as an autosomal dominant trait. The estimated prevalence ranges from 1 : 55.000 in England to 1 : 100.000 in Denmark. Skin-coloured to red-brownish papules are found on the predilection sites such as the chest, back, hairline, neck, scalp, and ears. Usually, the body folds such as arm pits, inguinal folds, and the skin below the breasts, are affected mildly. Lesions on hands and feet consist of small pits, spiky hyperkeratoses, verruca plana-like papules, and nail-changes (longitudinal white and/or red discolorations with a V-shaped splitting at the free margin). Small white papules may be found at the oral mucosa, and cause swelling of the salivary gland when these obstruct the salivary duct. The disease usually starts between the age of 6 and 10 years. Exacerbations occur after exposition to heat, sunlight, stress, and the use of lithium (14). A great interindividual variation exists in severity, varying from only minimal nail-changes to widespread forms covering a substantial part of the body surface. On histological examination acantholysis is found, due to disruption of the desmosome-keratin-filament complexes, as observed by electronmicroscopy. The underlying genetic defect has recently been localized on chromosome 12 (15,16). The acantholytic epidermal nevus probably represents a mosaic of Darier's disease, due to a somatic mutation of the Darier gene during embryogenesis. The classical treatment is systemic retinoids. However, the dosage has to be individualised.

In general the genetically determined dermatoses require lifelong care. Topical treatment remains the first line of maintenance therapy and aims at keratolysis and rehydration of the stratum corneum. Nevertheless, these classical topical treatments are insufficient as

monotherapy in the treatment of severe ichthyosis subtypes other than ADIV and Darier's disease. In these conditions, acitretin has been a major tool to improve various representants of ichthyosis and Darier's disease. The nosological independency of these conditions is reflected, at least to some extent, in the therapeutic response pattern to acitretin (thesis R. v Dooren (17)). In brief, the clinical responses to acitretin for NELI, ELI, CBIE, and Darier's disease, were respectively mild-excellent, excellent, excellent and moderate-excellent. The optimal daily dosage range (mg/day) fluctuated from respectively 10-55, 10-25, 40-60 to 10-60 respectively.
However, the toxicity potential of acitretin and the selective therapeutic action spectrum in these disorders require a further drug development programm. There is an urgent need to develop new treatments and to establish guidelines for these new approaches within the field of indications as heterogeneous as the spectrum of ichthyoses and Darier's disease. New therapeutical principles will be provided in part I of this thesis.

Palmoplantar keratodermas

The hereditary palmoplantar keratodermas (HPPK's) comprise a heterogeneous group of different clinical entities. HPPK may invalidate patients considerably (Figure 4).
The total group of HPPK is substantial although various entities may have a relatively rare occurrence. HPPK's may be restricted primarily to the hands and feet, or be associated with more generalized skin disorders, such as ADIV, CBIE, ELI, NELI, and erythrokeratoderma. Classification of the HPPK's restricted primarily to the hands and feet, is rather difficult, because of inter-and intraindividual variations and differences in nomenclature. Many authors have attempted to classify the HPPK's. Most classifications have been based on the morphology, distribution, associated symptoms and mode of inheritance. Despite the fact that these criteria are useful in making an attempt to classify the individual patient, previous classifications are of limited value, because they are rather dated. The most recent classification has appeared in 1986 in the literature (18). In recent years, a number of new entities have been defined (19-22). On the other hand, HPPK's which previously were considered to be distinct types, have been shown to be variants of the same type of HPPK (23,24). Furthermore, the underlying genetical defects have been localized in several types of HPPK. In HPPK of Vörner mutations were found in the gene coding for keratin 9 (25). In pachyonychia congenita mutations were detected in the keratin 6 or 16 gene (Jadassohn-Lewandowski type) (26) and in the keratin 17 gene (Jackson-Lawler type) (27). Three other HPPK's have been mapped: the Huriez syndrome to 4q28-31, focal acral hyperkeratosis of Costa to 2p and HPPK of Unna-Thost to 12q (28).

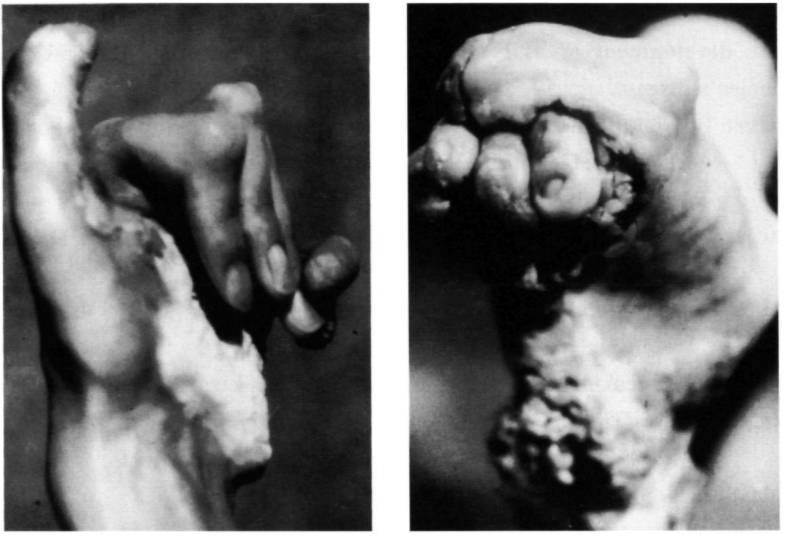


Fig. 4. The Omsted syndrome. Warty hyperkeratoses diffusely cover the palmoplantar skin leading to painful flexion contractures.

As classification is a prerequisite for choosing appropriate treatment and providing adequate genetic information, it is definitely warranted to develop an updated classification. It should be realised that the diagnosis of HPPK's is not an academic exercise but born out of practical relevance. In this respect the clinical appearance, histopathological hallmarks of a skin biopsy and a complete history and general examination of the patient are indispensable for a precise diagnosis. The diagnosis will inform on the natural course and most adequate therapeutical intervention.

In part 2 a description of previously recognized HPPK's will be given. These casuistical presentations are of interest because they highlight previously unrecognized presentations, and new therapeutical options for these forms of HPPK. Based on this information and a thorough study of the worlds literature, an integrated nosology for the HPPK's will be proposed.

Objectives of the thesis

(i) The development of a treatment program for patients with ichthyosis and Darier's disease.

target 1 **Establishment of the efficacy of new treatment modalities for ichthyoses and Darier's disease and the determination of disease specificity of new therapies.**

target 2 **Cytometric characterization of ichthyosis and Darier's disease before and after treatment.**

target 3 **An update of the therapeutic approach.**

(ii) The development of a nosology for palmoplantar keratodermas.

target 1 **Description of the expression of palmoplantar keratodermas and the construction of an integrated nosology for palmoplantar keratodermas.**

target 2 **Proposition for new therapeutical approaches.**

REFERENCES

1. Traupe H. The ichthyoses. A guide to clinical diagnosis, genetic counseling, and therapy. Springer-Verlag, Berlin 1989.
2. Steijlen PM. The monogenic disorders of keratinization. Classification and therapy. Thesis, Nijmegen, the Netherlands 1994.
3. Griffiths WAD, Leigh IM, Marks R. Disorders of keratinization. In: Champion RH, Burton JL, Ebling FJG, editors. Textbook of dermatology 5 th ed. Blackwell Scientific Publ. London 1992;1325-1390.
4. Braun-Falco O, Plewig G, Wolff HH. Keratosen. In: Braun-Falco O, Plewig G, Wolff HH, editors. Dermatologie und Venerologie 3 rd ed. Springer-Verlag Berlin 1984;470-487.
5. Williams ML, Elias PM. Genetically transmitted, generalized disorders of cornification. The ichthyoses. Dermatologic Clinics 1987;5:155-178.
6. Shapiro LJ, Weiss R, Webster D, France JT. X-linked ichthyosis due to steroid sulphatase deficiency. Lancet 1978;1:70-72.
7. DiGiovanna JJ, Bale SJ. Clinical heterogeneity in epidermolytic hyperkeratosis. Arch Dermatol 1994;130:1026-1035.
8. Kremer H, Zeeuwen P, McLean WHI, Mariman ECM, Lane EB, van de Kerkhof PCM, Ropers HH, Steijlen PM. Ichthyosis bullosa of Siemens is caused by mutations in the keratin 2e gene. J Invest Dermatol 1994;103:286-289.
9. McLean WHI, Morley SM, Lane EB, Eady RAJ, Griffiths WAD, Paige DG, Harper JI, Higgins C, Leigh IM. Ichthyosis bullosa of Siemens. A disease involving keratin 2e. J Invest Dermatol 1994;103:277-281.
10. Rothnagel JA, Traupe H, Wojcik S, Huber M, Hohl D, Pittelkow MR, Saeki H, Ishibashi Y, Roop DR. Mutations in the rod domain of keratin 2e in patients with ichthyosis bullosa of Siemens. Nature Genet 1994;7:485-490.

11. Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen SPM, Ponec M, Bon A, Lautenschl;ager S, Schorderet DF, Hohl D. Mutations of keratinocyte transglutaminase in lamellar ichthyosis. Science 1995;267:525-528.
12. De Laurenzi V, Rogers GR, Hamrock DJ, Marekov LN, Steinert PM, Compton JG, Markova N, Rizzo WB. Sjögren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. Nat Genet 1996;12:52-57.
13. Burge SM, Wilkinson WD. Darier-White disease: A review of the clinical features in 163 patients. J Am Acad Dermatol 1992;27:40-50.
14. Milton GP, Peck GL, Fu JL, DiGiovanna JJ, Nordlund JJ, Thomas JH, Sanders SF. Exacerbation of Darier's disease by lithium carbonate. J Am Acad Dermatol 1990;23:926-928.
15. Bashir R, Munro CS, Mason S, Stephenson A, Rees JL, Strachan T. Localisation of a gene for Darier;s disease. Hum Mol Genet 1993;11:1937-1939.
16. Richard G, Wright AR, Harris S, Doyle SZ, Korge B, Mazzanti C, Tanaka T, Harth W, McBride OW, Compton JG, Bale SJ, DiGiovanna JJ. Fine mapping of the Darier's disease locus on chromosome 12q. J Invest Dermatol 1994;103:665-668.
17. van Dooren-Greebe RJ. Methotrexate and acitretin in severe psoriasis and disorders of keratinization. Efficacy, side-effects and guidelines for long-term treatment. Thesis, Nijmegen, the Netherlands 1995.
18. Salamon T. An attempt at classification of inherited disorders of keratinization localized mainly, not exclusively on the palms and soles. Dermatol Monatsschr 1986;172:601-605.
19. Sybert VP, Dale BA, Holbrook KA. Palmoplantar keratoderma. A clinical, ultrastructural and biochemical study. J Am Acad Dermatol 1988;18:75-86.
20. Kastl I, Anton-Lamprecht I, Gamborg Nielsen P. Hereditary palmoplantar keratosis of the Gamborg Nielsen type. Clinical and ultrastructural characteristics of a new type of autosomal recessive palmoplantar keratosis. Arch Dermatol Res 1990;282:363-370.
21. Sharland M, Bleach NR, Goberdhan PD, Patton MA. Autosomal dominant palmoplantar hyperkeratosis and sensorineural deafness in three generations. J Med Genet 1992;29:50-52.
22. Dowd PM, Harman RRM, Black MM. Focal acral hyperkeratosis. Br J Dermatol 1983;109:97-103.
23. Küster W, Becker A. Indication for the identity of palmoplantar keratoderma type Unna-Thost with type Vörner. Thost's family revisited 100 years later. Acta Derm Venereol (Stockh) 1992;72:120-122.
24. Wachters DHJ. Over de verschillende morphologische vormen van de keratosis palmoplantaris, in het bijzonder over de keratosis palmoplantaris varians. Thesis, Leiden, the Netherlands 1963;49-67.
25. Reis A, Hennies H-C, Langbein L. Keratin 9 gene mutations in epidermolytic palmoplantar keratoderma (EPPK). Nature Genet 1994;6:174-179.
26. Mc Lean WHI, Rugg EL, Lunny DP. Keratin 16 and 17 mutations cause pachyonychia congenita. Nature Genet 1995;9:273-279
27. Bowden PE, Haley JL, Kansky A, Rothnagel JA, Jones DO, Turner RJ. Mutation of a type II keratin gene (K6a) in pachyonychia congenita. Nature Genet 1995;10:363.
28. Moss C, Savin J. The palmoplantar keratodermas. In: Moss C, Savin J, editors. Dermatology and the new genetics 1 st ed. Blackwell Science Ltd Oxford 1995;54-57.

Ichthyosis and Darier's disease

Part I

Introduction

As has been highlighted in the general introduction, the classical treatment for disorders of keratinization have their limitation with respect to efficacy and or side effects. Therefore, further development of new treatments is of importance. However, as each of these diseases are relatively seldom, new treatment development in the more frequently occurring polygenic disorders of keratinization (psoriasis spectrum) and inflammatory skin disorders such as acne, is an important source for new developments for ichthyosis and Darier's disease. In these frequently occurring diseases, major therapeutical progress has been made during the last decade. These developments were based on new leads in the pharmacological intervention with growth regulation of benign cell lines or neoplasias and interference with inflammation control.

A summary of the origin of new treatments with a potential impact for ichthyoses and Darier's disease is provided below. In the chapters of part I, an evaluation is provided whether a selection of these treatments might be of relevance for ichthyosis and Darier's disease.

Retinoids interfere with various aspects of epidermal growth and differentiation, as well as cutaneous inflammation. Epithelial tissues including epidermis, require a critical concentration of vitamin A for normal proliferation and differentiation. A deficiency or excess of vitamin A alter the normal pathways and lead to basal cell hyperproliferation and metaplasia. Retinoids can be considered both as growth promotors and as growth inhibitors, depending on the cell type and the experimental conditions. In quiescent epidermal cells, addition of retinoids induces increased proliferation. Such in contrast to the growth inhibition induced by retinoids in hyperproliferating (benign or malignant) cell lines, a feature that makes retinoids of interest in the treatment of hyperproliferative epidermal skin diseases and as antitumour drugs. As there is an inverse relationship in most tissues between proliferation and differentiation, retinoids inhibit differentiation of normal epidermal cells, whereas a stimulation of differentiation is observed in hyperproliferative tissues (1). These effects are believed to be primarily mediated by nuclear retinoic acid receptors, which are members of the steroid receptor superfamily (2). In addition to interference with epidermal behaviour, retinoids demonstrate immunomodulatory and anti-inflammatory effects (3).

As systemic treatment with acitretin has various side effects, the question arises whether a localized retinoid effect restricted to the target tissue can be reached by alternative principles. Theoretically, topical application and manipulation of retinoid metabolism are possible approaches to reach such. Many years ago, topical tretinoin, effective in the treatment of acne, has been shown to be effective to some extent in the treatment of ichthyosis (4) and Darier's disease (5). However, severe irritation of the skin prevented the routine use in these disorders of keratinization. Recently, topical 13-cis-retinoic acid

became available and has become a well established treatment for acne, either as systemical (6) or topical drug (7). As this new formulation has an ultra low irritancy potential, the question arose to what extent this new formulation might be beneficial in disorders of keratinization.

Imidazole derivatives have been found in recent years to inhibit several cytochrome P-450-dependent steps in the biosynthetic pathway of testicular, ovarian and adrenal steroids. Imidazole derivatives were originally used as antimycotic agents exerting their activity by inhibition of the fungal cytochrome P-450 enzyme dependent synthesis of ergosterol (8). After gynaecomastia developed in two patients treated with high doses for systemic mycotic disease, antiandrogenic effects of these drugs were detected (9). Thereafter, inhibition of testicular, ovarian and adrenal steroid metabolism by interference with the cytochrome P-450-dependent steps in their biosynthetic pathway was demonstrated (10-12). As a result, the therapeutic potential of imidazole derivatives was expanded with the treatment of androgen-dependent diseases such as prostatic cancer (13). One of the most recent imidazole derivatives is liarozole. Unlike first line imidazole derivatives, liarozole treatment of advanced prostatic cancer was not mediated via modulation of androgen metabolism. Remarkably, coexisting mucocutaneous side effects similar to those found in hypervitaminosis A could be attributed to increased plasma retinoic acid levels, which suggested an alternative pathway for liarozole to exert its cytotoxic effects (14). Furthermore, liarozole was found to inhibit the metabolism of endogeneously produced (15,16) or exogeneously applied (17,18) all-trans retinoic acid, resulting in increased levels of this vitamin in the skin. This was achieved by inhibition of the cytochrome P-450-mediated enzyme 4-hydroxylase, one of the main enzymes in the catabolic pathway of all-trans retinoic acid (19,20). Therefore, it was suggested that inhibitors of the all-trans-retinoic acid metabolism, might be useful in the treatment of skin conditions improving on retinoids, such as psoriasis and acne. Clinical improvement was obtained with oral liarozole in the treatment of psoriasis, whereas plasma testosterone and cortisol levels remained in the normal range (21). Consequently, the question arose, whether this new imidazole compound could be effective in monogenic disorders of keratinization. As retinoids are effective in both systemic and topical formulations, an additional question was whether liarozole might be effective as topical treatment for monogenic disorders of keratinization.

Calcipotriol is a synthetic vitamin D3 analogue with a high binding affinity to the cellular receptor for the biologically active form of vitamin D3: 1,25 dihydroxyvitamin D3 (calcitriol) (22). Like retinoids, vitamin D3 analogues mediate their biological response by binding to its nuclear receptor, also being a member of the steroid receptor superfamily (23). A wide variety of cells posess nuclear receptors for vitamin D3, including keratinocytes and fibroblasts (24,25). Calcipotriol and calcitriol, the naturally

occuring active form of vitamin D3 produce an equivalent dose-dependent inhibition of proliferation, and stimulation of terminal differentiation in cultured human keratinocytes (24-30). Calcipotriol was found effective in the treatment of the polygenic disorders of keratinization, psoriasis (25,31,32) and pityriasis rubra pilaris (33). The question was addressed whether calcipotriol might be of benefit in the treatment of monogenic disorders of keratinization.

REFERENCES

1. Vahlquist A, Törmä H. Retinoids and keratinization. Current concepts. Int J Dermatol 1988;2:81-95.
2. Darmon M, Blumenberg M. Retinoic acid in epithelial and epidermal differentiation. In: Molecular biology of the skin: The keratinocyte. Academic press inc. 1993:181-206.
3. Pilkington T, Brogden RN. Acitretin. A review of its pharmacology and therapeutic use. Drugs 1992;43:597-627.
4. Muller SA, Belcher RW, Esterly NB, Lochner JC, Miller JS, Roenigk H, Weismann L. Keratinizing dermatoses. Combined data from four centres, on short-term topical treatment with tretinoin. Arch Dermatol 1977;113:1052-1054.
5. Fulton JE, Gross PR, Cornelius CE, Kligman AM. Darier's disease. Treatment with topical vitamin A acid. Arch Dermatol 1968;98:396-399.
6. Peck OL, Olsen TG, Butkus D. Isotretinoin versus placebo in the treatment of cyctic acne. J Am Acad Dermatol 1982;6:735-745.
7. Chalker DK, lesher JL, Smith JG. Efficacy of topical isotretinoin 0.05% gel in acne vulgaris.: Results of a multicenter, double-blind investigation. J Am Acad Dermatol 1987;17:251-254.
8. Restrepo A, Stevens DA, Utz JP. First International Symposium on Ketoconazole. Rev Infect Dis 1980; 2:519-699.
9. De Felice R, Johnson DG, Galgiani JN. Gynecomastia with Ketoconazole. Antimicrob Agenst Chemother 1981; 19:1073-1074.
10. Pont A, Williams PL, Azhar S, Reitz RE, Bochra C, Smith ER. Ketoconazole blocks testoterone synthesis. Arch Intern Med. 1982; 142:2137-40.
11. Pont A, Williams PL, Loose DS, Feldman D, Reitz RE, Bochra C. Ketoconazole blocks adrenal steroid synthesis. Ann Intern Med 1982; 97:370-2.
12. De Coster R, Caers I, Coene MC, Amery W, Beerens D, Haelterman C. Effects of ketoconazole therapy on the main plasmatesticular and adrenal steroids in previously untreated prostatic cancer patients. Clin Endocrinol (Oxf) 1986; 24:657-64.
13. Trachtenberg J, Halpern N, Pont A. Ketoconazole: a novel and rapid treatment for advanced prostatic cancer. J. Urol 1983; 130:152-3.
14. Mahler C, Verhelst J, Denis L. Ketoconazole and liarozole in the treatment of advanced prostatic cancer. Cancer 1993; 71:suppl:1068-73.
15. De Coster R, van Ginckel R, van Moorselaar RJA, et al. Antitumoral effect of R 75251 in prostatic carcinoma: experimental and clinical studies. Proc. Am. Assoc. Cancer Res. 1991;32:213.
16. van Ginckel R, de Coster R, Wouters W, et al. Antitumoral effects of R75251 on the growth of transplantable R3327 prostatic adenocarcinoma in rats. Prostate 1990;16:313-23.

17. Wouters W, van Dun J, Dillen A, Coene M, Cools W, de Coster R. Effects of liarozole, a new antitumoral compound, on retinoic acid induced inhibition of cell growth and on retinoic acid metabolism in MCF-7 human breast cancer cells. Cancer-Res 1992;52:2841-6.
18. Williams JB, Napoli JL. Metabolism of retinoic acid and retinol during differentiation of F9 embryonal carcinoma cells. Proc Natl Acad Sci USA 1985;82:4658-62.
19. van Wauwe J, Coene MC, Goossens J, van Nijen G, Cools W, Lauwers W. Effects of cytochrome P-450 inhibition on the in vivo metabolism of all-trans-retinoic acid in rats. J Pharmac Exp Ther 1990;252:365-9.
20. Bruynseels J, Coster R de, Rooy P van, et al. R 75251, a new inhibitor of steroid biosynthesis. Prostate 1990;16:345-357.
21. Dockx P, Decree J, Degreef H. Inhibition of the metabolism of endogenous retinoic acid as treatment for severe psoriasis: an open study with oral liarozole. Br J Dermatol 1995;133:426-432.
22. Binderup L, Bramm E. Effects of a novel vitamin D analogue MC 903 on cell proliferation and differentiation in vitro and calcium metabolism in vivo. Biochem Pharmacol 1988;37:889-95
23. Murdoch D, Clissold SP. Calcipotriol. A review of its pharmacological properties and therapeutic use in psoriasis vulgaris. Drugs 1992;43:415-429.
24. Clemens TL, Adams JS, Horiuchi N et al. Interaction of 1,25-dihydroxyvitamin D3 with keratinocytes and fibroblasts from skin of normal subjects and a subject with vitamin D dependent rickets, type II: A model for study of mode of action of 1,25- dihydroxyvitamin D3. J Clin Endocrinal Metab 1983;56:824-30
25. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. J Am Acad Dermatol 1988;19:516-28
26. Smith EL, Walworth NC, Holick MF. Effect of 1,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum free conditions. J Invest Dermatol 1986;86:709-14
27. Kondo S, Satoh Y, Yamaguchi J. The inhibitory effects of 1,25-dihydroxyvitamin D3 on DNA synthesis in cultured epidermal cells from patients with psoriasis vulgaris. Nishi Hihon J Derm 1988;50:679
28. Kragballe K. MC 903, a non-calciotropic vitamin D3 analogue stimulates differentiation and inhibits proliferation of cultured human keratinocytes. J Invest Dermatol 1988;91:383
29. Holick MF, Smith E, Pincus S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxyvitamin D3. Arch Dermatol 1987;123:1677-1683
30. Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T. Regulation of terminal differentiation of culture mouse epidermal cells by 1,25-dihydroxyvitamin D3. Endocrinology 1983;3:1950-57
31. Kragballe K. Treatment of psoriasis by the topical application of the novel vitamin D3 analogue MC 903. Arch Dermatol 1989;125:1647-52
32. Kragballe K, Gjertsen BT, de Hoop D et al. Double-blind, right-left comparison of calcipotriol and betamethasone valerate in treatment of psoriasis vulgaris. Lancet 1991;337:193-6.
33. Van de Kerkhof PCM, de Jong EMGJ. Topical treatment with the vitamin D3 analogue MC903 improves pityriasis rubra pilaris: clinical and immunohistochemical observations. Br J Dermatol 1991;125:293-294

Chapter 1

Topical treatment of ichthyoses with 13-cis-retinoic acid. A clinical and immunohistochemical study.

GPH Lucker
PCM van de Kerkhof
FACM Castelijns
MR van Dijk
LJM Schalkwijk
PM Steijlen

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SUMMARY

In a prospective, double blind, bilaterally paired comparative study, a cream containing 13-cis-retinoic acid (13-cis-RA) 0.1% and the cream base only were applied during a period varying between 4 and 10 weeks in 12 ichthyosis patients (1 patient with autosomal dominant ichthyosis vulgaris (ADIV), 7 patients with X-linked recessive ichthyosis (XRI), 2 patients with bullous congenital ichthyotic erythroderma of Brocq (BCIE), and 2 patients with erythrodermic lamellar ichthyosis (ELI)). A significant unilateral improvement was found for the clinical parameters scaling and induration. Clinical improvement was observed in all ichthyosis groups, except for ADIV. Continuation of the treatment beyond the first month, caused a further reduction of skin lesions. Side effects due to the study medication were only minimal and easily controlled by adjusting the application frequency. Biopsies for immunohistochemical examination were taken from representative skin lesions from 9 patients, one before and one from each side after treatment. The keratins 4, 13, and 8, were induced by treatment with topical 13-cis-RA. These keratins could not be detected in the biopsies taken before treatment nor in the biopsies derived from the cream-base treated side. Keratin 4 was induced in 6 of the 9 patients. Induction of keratin 13, visualized with mab 1C7 and 2D7, was found in 1 and 3 patients respectively. Keratin 8, visualized with mab LE4.1 and M20, was induced in respectively 1 and 3 patients. No clear correlation could be established between expression of keratins and clinical efficacy. Cellular proliferation tended to be increased at the 13-cis-RA treated side. No changes were found for the investigated parameters of inflammation.

INTRODUCTION

Oral retinoids have proven to be very effective in the treatment of ichthyosis (1-3). Because of side effects, caused by systemic long term therapy with retinoids, topical preparations have regained much of their former importance. Common topical treatment modalities are based on keratolysis and hydration of the epidermis. Although some improvement may be achieved, severe ichthyosis subtypes do not improve sufficiently using these topical treatments.

Topical retinoids affect epidermal proliferentiation and differentiation (4). Good responses have been reported in patients with lamellar ichthyosis, ichthyosis vulgaris and to a lesser extent in patients with X-linked ichthyosis and epidermolytic hyperkeratosis, treated with topical tretinoin 0.1% cream. Adverse reactions were erythema, pruritus and irritation and occured in 26 of the 30 patients (87%) (5). Because of these irritative side effects, the use of topical retinoids has been limited (6).

Recently, Steijlen et al. treated one patient with autosomal dominant ichthyosis vulgaris, and seven patients with non erythrodermic lamellar ichthyosis (NELI), for four weeks using a double-blind, bilaterally paired approach with topical 13-cis-retinoic acid (13-cis-RA) 0.1% and cream base only. A good clinical improvement, in favour of the verum

treated side, was observed in two patients with NELI. Immunohistochemical examination revealed induction of keratin 4 in two NELI patients (7). It was suggested that the selective modulation of the cytokeratin pattern might provide an immunohistochemical tool to investigate the mode of action of retinoids.

The aim of the present study was an investigation of the clinical efficacy and tolerability of prolonged topical treatment with 13-cis-RA in one patient with autosomal dominant ichthyosis vulgaris (ADIV), seven patients with X-linked recessive ichthyosis (XRI), 2 patients with bullous congenital ichthyotic erythroderma of Brocq (BCIE) and 2 patients with erythrodermic lamellar ichthyosis (ELI). Furthermore, immunohistochemical assessment was made of parameters of proliferation, keratinization and inflammation, using monoclonal antibodies, to examine whether a selective modulation of the cytokeratin pattern could be established.

PATIENTS AND METHODS

Patients

One patient with autosomal dominant ichthyosis vulgaris (ADIV), seven with X-linked recessive ichthyosis (XRI), two with bullous congenital ichthyotic erythroderma of Brocq (BCIE) and two patients with erythrodermic lamellar ichthyosis (ELI) were treated with 13-cis-RA after informed consent was obtained. The XRI-patients had a documented steroidsulphatase deficiency. NELI- and BCIE-patients had characteristic changes on light- and electronmicroscopic examination. Further details regarding ichthyosis subtype, sex, age, duration of treatment, reason for discontinuation and previous treatments are summarized in Table I.

Topical as well as systemic treatment was stopped respectively 2 and 4 weeks before the trial was started. Using a prospective, bilaterally paired comparative, double-blind approach, 13-cis-RA 0.1% in cream base and cream base only (Hoffmann-la Roche, Basel, Switzerland) were applied twice daily, without occlusion, on a rectangular area of 10 by 10 cm on 2 symmetrical and representative lesions. The creams were applied thinly and evenly during a test period varying between 4 and 10 weeks. In the event of intolerable irritation, patients were instructed to reduce the treatment frequency. In order to establish clinical efficacy, scaling, erythema, and induration were scored using a 4 point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Statistical analysis was carried out using the Wilcoxon signed-rank test for paired data.

Analytical procedures

Biopsies from representative skin lesions within the test areas were taken from 9 patients, one at the start and one from each area at the end of treatment. The keratin antibodies used were the same as used previously (7). In addition the monoclonal antibody Ki-67 was used to assess proliferation, whereas Leu14, OKT6, WT14, T11, and anti-elastase were used to assess B-lymphocytes, Langerhans cells, macrophages and monocytes, T-

lymphocytes, and polymorphonuclear leucocytes respectively. Biopsy procedures and immunohistochemical staining methods were used as described before (7). An indirect alkalic phosphatase technique was used for staining with the antibodies Ki-67 and anti-elastase.

Ki-67 positive nuclei were counted per millimetre length of section. Presence in the epidermis and dermis of the remaining stained structures were assessed using a semi-quantitative scale:

Epidermis: 0= no staining; 1= sporadic staining; 2= minimal staining; 3= moderate staining; 4= moderate-pronounced staining; 5= pronounced staining; 6= whole epidermis stained.

Dermis: 0= absence; 1= sporadically present; 2= 1-25% of infiltrate cells stained; 3= 26-50% of infiltrate cells stained; 4= 51-75% of infiltrate cells stained; 5= 76-99% of infiltrate cells stained; 6= 100% of infiltrate stained.

Table I. Patient details

patient	diagnosis	sex	age	duration of treatment (weeks)	reason for discontinuation	previous treatments
1	ADIV	v	26	10	end of study	urea
2	XRI	m	53	6	end of study*	propylaenglycol
3	XRI	m	17	10	end of study	salicylic acid, urea, ac. lacticum, propylaenglycol
4	XRI	m	30	4	clearance	urea
5	XRI	m	32	6	clearance	urea
6	XRI	m	45	10	end of study	salicylic acid, urea, petrolatum
7	XRI	m	17	10	end of study	none
8	XRI	m	62	10	end of study	salicylic acid, petrolatum unknown
9	BCIE	m	25	10	end of study	etretinate, petrolatum
10	BCIE	f	39	9	end of study*	ac. lacticum, urea,
11	ELI	f	51	8	end of study*	petrolatum etretinate, urea,
12	ELI	f	37	7	holidays	ac.lacticum

* Patients used up all the trial medication

RESULTS

Clinical response

Patient details and side effects of the treatment with 13-cis-RA, are summarized in Table I. Table II overviews the clinical efficacy of both verum and cream base.

At the end of the study, 6 of the 12 treated patients showed a half side reduction of scaling in favour of the 13-cis-RA treated side (patient no. 2,7,8,9,11,12)(Figs. 1 and 2).

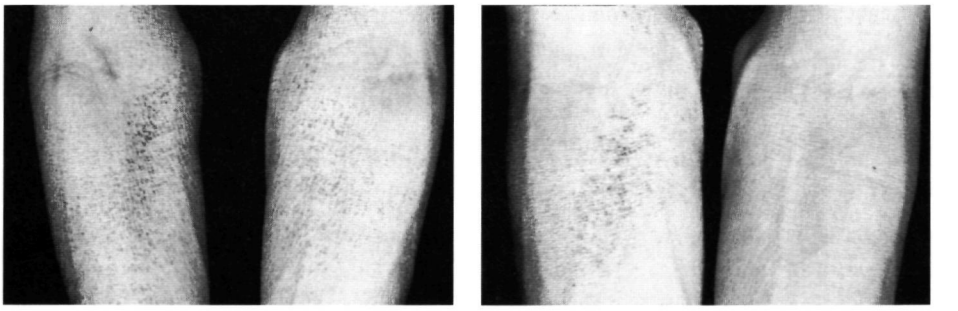

Fig. 1 a. XRI-patient with the characteristic phenotype consisting of small brown scales covering the arms with sparing of the flexures, before treatment. **1b.** After treatment, the 13-cis-RA treated left side has improved markedly, whereas only slight improvement is observed on the cream base treated right side.

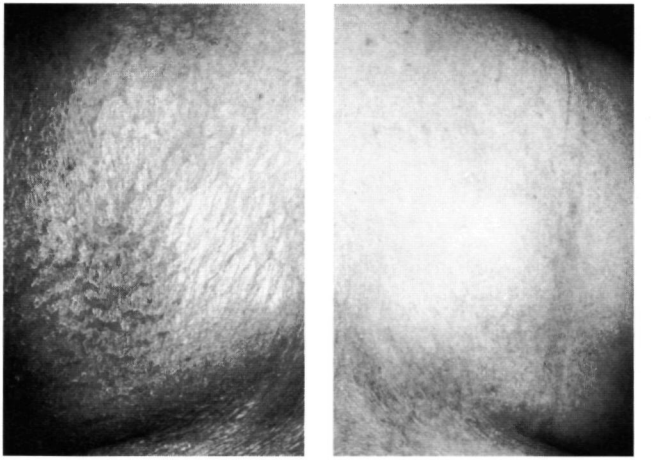

Fig. 2 After treatment, substantial improvement is seen on the 13-cis-RA treated right side. Scaling is almost completely absent, whereas minimal erythema persists. The scaling on the cream base treated left side, is comparable with the pretreatment state. Erythema is clearly visible, due to absence of reflections in this close-up view.

Table II. Clinical effects of topical 13-cis-RA

patient	scaling				induration			
	vehicle		13-cis-RA		vehicle		13-cis-RA	
	start	end	start	end	start	end	start	end
1	2	2	2	2	2	1	2	1
2	3	3	3	1	3	3	3	1
3	2	1	2	2	1	0	1	0
4	2	0	2	0	1	0	1	0
5	1	0	1	0	1	0	1	0
6	3	1	3	1	2	0	2	0
7	3	3	3	0	1	1	1	1
8	2	1	2	0	1	1	1	0
9	3	2	3	0	3	2	3	0
10	3	1	3	2	3	2	3	2
11	3	3	3	0	2	3	2	0
12	3	3	3	1	3	3	3	1

In the subgroup with ELI, all patients showed unilateral improvement, whereas in the XRI and BCIE subgroups a half side reduction was achieved in respectively 3 out of 7 and 1 out of 2 patients. No clinical improvement was achieved in the patient with ADIV. Five of these responders also showed improvement of induration (patient 2,8,9,11,12). The reduction of scaling at the 13-cis-RA side, compared to the cream base side, was statistically significant ($p<0.05$), as was the reduction of induration ($p<0.05$). No significant alterations were observed in erythema.

An interindividual variation was observed with regard to duration of treatment needed to achieve a half side reduction. The earliest response was noticed within one week of treatment, while the latest response was not observed before eight weeks of treatment. Within the first month of treatment, a half side reduction was achieved in 5 patients (patient no. 2,7,9,11,12). In all of them further improvement was obtained by continuing the treatment beyond the first month.

Itching and irritation of the skin (burning), were reported as side effects in four patients (33%) at the verum treated side only. These side effects, though, were only minimal and could be easily controlled by adjusting the application frequency. Irritation did not necessitate discontinuation of the treatment in any patient.

Histological findings

Before treatment RKSE60, recognizing keratin 10, marker for cornifying epithelia, reacted with the suprabasal compartment in a homogeneous pattern. Treatment with topical 13-cis-RA did not change the reaction pattern. The basal cell markers RCK102

and RCK107, stained the basal cell layers in all biopsies taken before and after treatment. No consistent change in staining patterns was observed after treatment with 13-cis-RA. The markers for non-cornifying epithelia, i.e. antibodies 6B10 to keratin 4, and 1C7 and 2D7 to keratin 13, showed no reaction in the biopsies taken before treatment. After treatment with 13-cis-RA a significant increase of keratin 4 expression (Mab 6B10) was observed ($p<0.05$). Staining for keratin 4 was absent at baseline, but was present in 6 out of the 9 patients after treatment: staining was moderate in 1 BCIE (Fig. 3) and 1 ELI, minimal in 1 ELI, and sporadic in 3 XRI patients (Table III). Staining for keratin 13 was sporadic in 1 BCIE patient, using mab 1C7, and sporadic in 1 ADIV, 1 BCIE, and 1 ELI patient, using mab 2D7. Induction of keratin 4 and 13, was only found at the 13-cis-RA treated side. The markers for simple epithelial cells, i.e. antibodies to keratin 7, 18, and 19, showed no epidermal staining in the biopsies taken either before or after treatment. Staining for keratin 8, using the Mab CAM5.2, M20, and LE4.1, was absent at baseline. After treatment, minimal staining of Mab M20 was found in 1 ADIV, 1 XRI, and 1 ELI patient (Fig. 4), whereas Mab LE4.1 stained sporadic in 1 ELI patient (Fig. 5), at the 13-cis-RA treated side only. No staining was found with CAM5.2. For Mab KS8.12, staining keratin 13 and proliferation-associated keratin 16, no consistent change in staining pattern was observed after treatment with 13-cis-RA. The average number of cycling cells per millimetre epidermis was 103 before treatment. After treatment, this number was 120 at the 13-cis-RA treated side, and 90 at the cream-base treated side. No changes were found for the investigated parameters of inflammation.

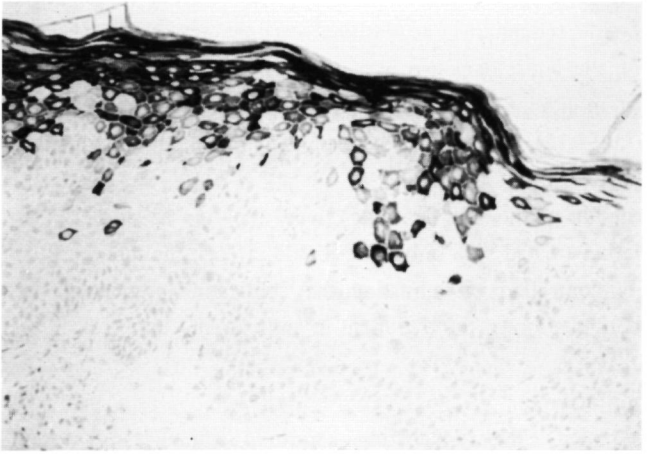

Fig 3. Induction of keratin 4 visualized with mab 6B10 in a patient with BCIE at the 13-cis-RA treated side. Keratin 4 expression is only found in the upper epidermal cell layers.

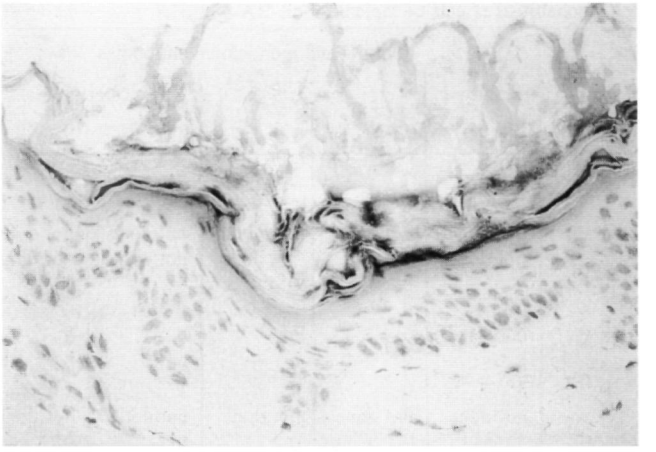

Fig. 4. Minimal staining with mab M20, indicating keratin 8, in the ELI patient at the 13-cis-RA treated side. Induction of keratin 8 is confined to the upper granular cell and cornified cell layers.

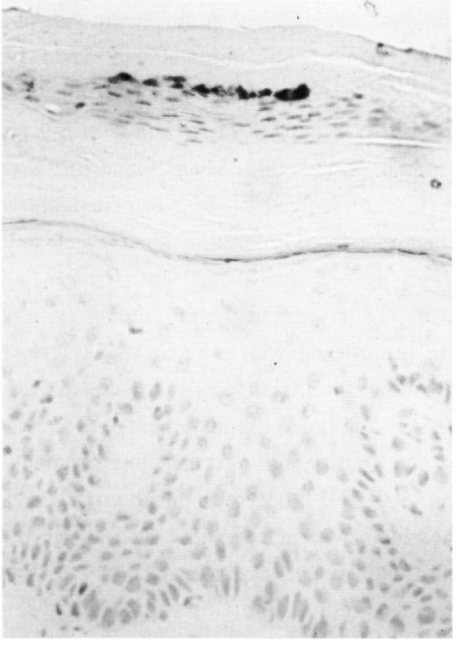

Fig. 5. Same patient as Figure 4, showing expression of mab LE4.1, staining keratin 8. Induction of keratin 8 is restricted to the upper parakeratotic layers of the stratum corneum.

Table III. Immunohistochemical effects of topical 13-cis-RA

patient	keratins and monoclonal antibodies				
	keratin 4	keratin 8		keratin 13	
	6 B 10	M 20	LE 4.1	1 C 7	2 D 7
1	0	2	0	0	1
2	1	0	0	0	0
3	not done	not done	not done	not done	not done
4	not done	not done	not done	not done	not done
5	1	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	1	2	0	0	0
9	3	0	0	1	1
10	not done	not done	not done	not done	not done
11	2	0	0	0	0
12	3	2	1	0	1

DISCUSSION

Twelve ichthyosis patients were treated in a prospective, double-blind, bilaterally paired study, with a cream containing 13-cis-retinoic acid 0.1% and its cream-base only, for a period varying between 4 and 10 weeks. A statistically significant improvement of scaling and induration was observed. Clinical improvement was observed in all ichthyosis groups, except for ADIV. Irritation was minimal and could be controlled easily by adjustment of the application frequency. Immunohistochemical assessment of biopsies, revealed a statistically significant induction of keratin 4 in 6/9 patients. Keratin 13 was induced in 3/9 patients. Induction of keratin 8 was found in 3/9 patients. These keratins were only detected at the 13-cis-RA treated side. No induction was observed at baseline. Mab Ki-67, taken as a parameter for proliferation was elevated after treatment at the 13-cis-RA treated side.

The good results with respect to efficacy and tolerability of topical 13-cis-RA in ichthyosis patients, are in accordance with a previous case report on 2 patients with NELI (7), and demonstrates that other ichthyosis subgroups as XRI, BCIE, and ELI, may also improve on 13-cis-RA. Additional improvement was observed by extending the treatment period beyond the first month. Further improvement may be achieved by adapting the concentration (8,9), the mode of application (10) and the cream basis (11). It is attractive to hypothesize that other retinoids, such as the arotinoids, may be even more effective as a topical treatment for ichthyosis (12).

Keratin 4 and 13, are foetal keratins absent in normal adult skin. Induction has been described after treatment with topical retinoids (7,13,14). This selective modulation of the keratin pattern has been proposed as an immunohistochemical tool to investigate the mode of action of retinoids (7). Our immunohistochemical results are in conjunction with these previous observations. Furthermore, we found selective induction of keratin 8 at the

13-cis-RA treated skin, which to our knowledge, has not been described previously. In adult skin, keratin 8 is normally expressed in apocrine and eccrine sweat glands (15). In fetal skin it is expressed in the basal cell layer (16). It is possible that keratin 8 represents another keratin, selectively modulated by retinoids. As fetal keratins are normally not expressed during epidermal differentiation, one could argue about the relevance of their induction. No clear correlation could be established between the extent of expression and the clinical efficacy to 13-cis-RA.

In conclusion, topical treatment of ichthyoses with 13-cis-RA 0.1% was followed by a clinical improvement in XRI, BCIE and ELI. Irritation of the skin was minimal and could be controlled easily. In addition to keratin 4 and 13, keratin 8 might represent another keratin induced by retinoids, and thus provide a new immunohistochemical tool to investigate the mode of action of retinoids. The clinical relevance of this keratin expression remains to be established.

ACKNOWLEDGEMENTS

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REFERENCES

1. Baden HP, Buxman MM, Weinstein GD, Yoder FW. Treatment of ichthyosis with isotretinoin. J Am Acad Dermatol 1982;6:716-720.
2. Happle R, van de Kerkhof PCM, Traupe H. Retinoids in disorders of keratinization:their use in adults. Dermatologica 1987;175:107-124.
3. Geiger JM, Czarnetzki BM. Acitretin (RO 10-1670, Etretin): overall evaluation of clinical studies. Dermatologica 1988;176:182-190.
4. Plewig G, Wolff HH, Braun-Falco O. Lokalbehandlung normaler und pathologischer menschlicher Haut mit Vitamin-A-Säure. Klinische, histologische und elektronenmikroskopische Untersuchungen. Arch Klin Exp Dermatol 1971;239:390-413.
5. Muller SA, Belcher RW, Esterly NB, Lochner JC, Miller JS, Roenigk H, Weissman L. Keratinizing dermatoses. Combined data from four centers on short-term topical treatment with tretinoin. Arch Dermatol 1977;113:1052-1054.
6. Orfanos CE, Ehlert R, Gollnick H. The retinoids, a review of their clinical pharmacology. Drugs 1987;34:459-503.
7. Steijlen PM, Reifenschweiler DOH, Ramaekers FCS, van Muijen GNP, Happle R, Link M, Ruiter DJ, van de Kerkhof PCM. Topical treatment of ichthyoses and Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study. Arch Dermatol Res 1993;285:221-226.
8. Plewig G, Ruhfus A, Klövekorn W. Sebum suppression after topical application of retinoids (arotinoid and isotretinoin). J Invest Dermatol 1983;80:357.
9. Elbaum DJ. Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne. J Am Acad Dermatol 1988;19:486-491.
10. Fulton JE, Gross PR, Cornelius CE, Kligman AM. Darier's disease. Treatment with topical vitamin A acid. Arch Derm 1968;98:396-399.

11. Lehman PA, Slattery JT, Franz TJ. Percutaneous absorption of retinoids: influence of vehicle, light exposure and dose. J Invest Dermatol 1988;91:56-61.
12. Chung JC, Law MYL, Elliott ST, Elias PM. Diazacholesterol-induced ichthyosis in the hairless mouse. Assay for comparative potency of topical retinoids. Arch Dermatol 1984;120:342-347.
13. Rosenthal DS, Griffiths EM, Yuspa SH, et al. Acute or chronic topical retinoic acid treatment of human skin in vivo alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10, and 14. J Invest Dermatol 1992;98:343-350.
14. Steijlen PM, Happle R, Muijen GNP van, et al. Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. Dermatologica 1991;182:178-183.
15. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31:11-24.
16. Muijen van GNP, Warnaar SO, Ponec M. Differentiation-related changes of cytokeratin expression in cultured keratinocytes and in fetal, newborn, and adult epidermis. Exp Cell Res 1987;171:331-345.

Chapter 2

Effect of topical calcipotriol on congenital ichthyoses.

GPH Lucker
PCM van de Kerkhof
MR van Dijk
PM Steijlen

Br J Dermatol 1994;131:546-550

SUMMARY

We investigated the clinical efficacy of topically applied calcipotriol in 6 patients with congenital ichthyosis, using a double-blind, bilaterally paired, comparative approach. Unilateral improvement, in favour of the calcipotriol-treated side, was observed in 3 patients with lamellar ichthyosis. A beneficial response was also observed in a patient with bullous ichthyotic erythroderma of Brocq. No clinical side-effects or laboratory anomalies were observed. This study indicates that calcipotriol constitutes a new and promising approach in alleviating disorders of keratinization characterized by hyperproliferation, other than psoriasis.

INTRODUCTION

Lamellar as well as epidermolytic ichthyoses, comprise congenital ichthyosis subgroups, not associated with other diseases. The lamellar ichthyoses are a heterogeneous group of at least five types inherited as autosomal recessive traits, and an autosomal dominant type. Among the autosomal recessive types, erythrodermic and non-erythrodermic subtypes can be distinguished, i.e. erythrodermic autosomal recessive lamellar ichthyosis (EARLI) and non-erythrodermic autosomal recessive lamellar ichthyosis (NEARLI). The group of epidermolytic ichthyoses comprises at least three different autosomal dominant diseases: bullous ichthyotic erythroderma of Brocq, ichthyosis bullosa of Siemens, and ichthyosis hystrix of Curth-Macklin. The Brocq and Siemens types comprise the bullous ichthyoses, in which the occurrence of blisters enables differentiation from the Curth-Macklin type. The Comèl-Netherton syndrome is a rare syndrome of congenital ichthyosis linearis circumflexa associated with trichorrhexis invaginata [1].

Common treatment modalities, based on keratolysis and (re)hydration of the epidermis, are useful in the treatment of autosomal dominant ichthyosis vulgaris (ADIV), but severely affected X-linked recessive (XRI), lamellar, and bullous ichthyosis patients can only be improved by the use of oral retinoids. Because of the potential toxicity of systemic retinoids, it would be an advantage if an effective topical agent was available.

Calcipotriol is a vitamin D3 analogue which has a high binding affinity to the cellular receptor for the biologically active form of vitamin D3: 1,25 dihydroxyvitamin D3 (calcitriol) [2]. Receptors for calcitriol have been demonstrated in various cells, including keratinocytes and fibroblasts [3,4]. Calcitriol and calcipotriol produce equivalent dose-dependent inhibition of proliferation [5-7], and stimulation of terminal differentiation [3,4,7,8], in cultured human keratinocytes [9].

Favourable results in the treatment of psoriasis have been obtained with calcitriol [10], and calcipotriol [4,11,12], and a beneficial effect was also noted in a case of pityriasis rubra pilaris treated with calcipotriol [13]. Extensive clinical research on topical calcipotriol has not revealed any significant rise in serum calcium, or increase in urine calcium excretion, in patients using less than 100 g per week [14]. The present study was

performed to assess the clinical efficacy of topical calcipotriol in the treatment of lamellar and epidermolytic ichthyoses, and a case of Comèl-Netherton syndrome.

PATIENTS AND METHODS

In a prospective, double-blind, bilaterally paired comparative study, six patients with congenital ichthyosis were treated with calcipotriol ointment (50 μg/g) and the ointment vehicle. Details of ichthyosis subgroup, sex, age, duration of treatment and previous treatment are summarized in Table I. All affected body areas with the exception of the face, scalp and genital region, were treated with the study medication. Other topical and systemic treatments were stopped 2 and 10 weeks, respectively, before the start of the study. Patients were allowed to apply the emollient cream Locobase (Gist-brocades nv Delft, the Netherlands), to the affected body areas during the wash-out phase, and to the face and genital region throughout the study period. The ointments were applied thinly and evenly, twice daily, for a period of 12 weeks. A maximum amount of 120 g/week was dispensed for each side of the body. To avoid inadvertent spread to other body areas, patients were instructed to wash their hands after application of the ointments. Blood samples for haematological and biochemical analysis were taken 2 weeks before the start of the study, and at weeks 2 and 12 of the treatment phase. Serum calcium levels were monitored, but urinary calcium excretion was not assessed in this study. The clinical assessment was performed using a four-point scale for scaling, roughness and erythema.

Table I. Patient details

patient	diagnose	sex	age	duration of treatment	previous treatment
1	NEARLI	M	25	12 weeks	Acitretin, urea, sal. acid
2	NEARLI	M	21	12 weeks	Acitretin, urea, sal.acid, lactic acid
3	EARLI	F	45	12 weeks	Acitretin, retinoic acid, sal.acid
4	Siemens	M	56	12 weeks	Acitretin
5	Brocq	F	39	12 weeks	Acitretin, sal.acid, urea, 13-cis-retinoic acid
6	Netherton	F	18	12 weeks	Urea, sal.acid

RESULTS

The clinical effects of calcipotriol ointment and the ointment vehicle are summarized in Table II. At the end of the study, there was a reduction of scaling and roughness on the calcipotriol-treated side in all three patients with lamellar ichthyosis (Figs 1-3), and in the patient with bullous ichthyotic erythroderma of Brocq. The Comèl-Netherton patient showed unilateral improvement with regard to roughness. The degree of erythema remained constant during the study in all patients, except for the patient with the Comèl-Netherton syndrome, who showed worsening on the ointment base-treated side at the end

of the study. The patient with ichthyosis bullosa of Siemens did not show any change in severity on either side of the body.
Improvement on the ointment base-treated side was seen in one responder, but to a lesser extent than the calcipotriol-treated side, and one patient showed equal improvement on both sides.
Apart from some discomfort on both sides, attributable to the fatty ointment base, no unilateral side-effects were reported. Blood parameters remained within the normal range throughout the study. Roughness and scaling appeared to be the best parameters to establish clinical improvement.

Table II. Clinical effects of topical calcipotriol

	vehicle		calcipotriol	
patient no.	start	end	start	end
Effect on scaling	2	2	2	1
1	3	2	3	1
2	3	3	3	1
3	2	1	2	1
4	3	3	3	1
5	2	2	3	3
6				
Effect on roughness				
1	2	2	2	1
2	3	2	3	1
3	3	3	3	1
4	2	1	2	1
5	3	3	3	1
6	2	2	3	2
Effect on erythema				
1	2	0	0	0
2	3	0	0	0
3	3	2	2	2
4	2	0	0	0
5	3	1	1	1
6	2	2	2	2

0, absent; 1, slight; 2, moderate; 3, severe

DISCUSSION

This study is the first to demonstrate a substantial improvement of lamellar and epidermolytic ichthyoses with topical calcipotriol therapy. No clinical side-effects or laboratory anomalies were found in our study, in particular, there was no rise in serum calcium.

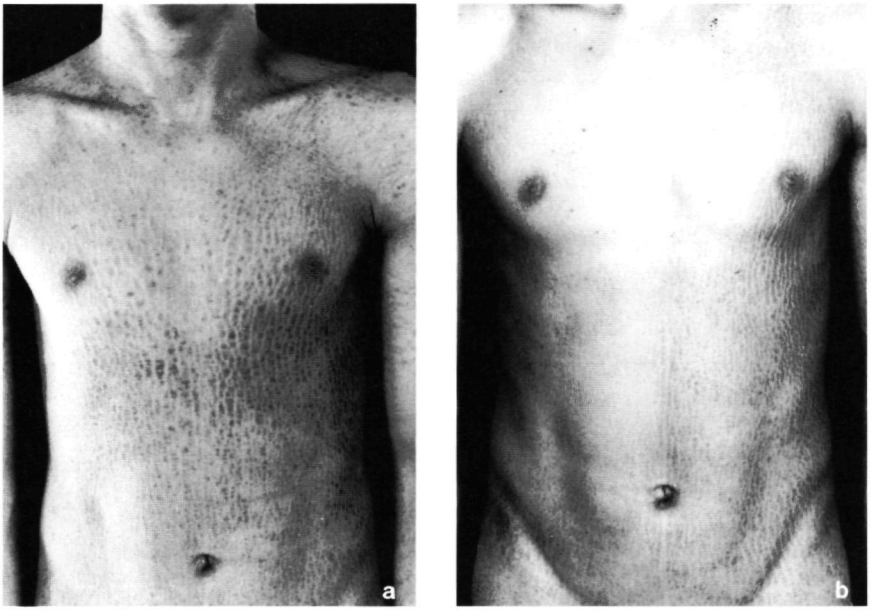

Fig. 1 a. NEARLI patient with the typical phenotype consisting of large brown scales covering the trunk, before treatment. **b.** After treatment, the calcipotriol-treated right side has improved markedly, whereas only slight improvement has occurred on the ointment base-treated left side.

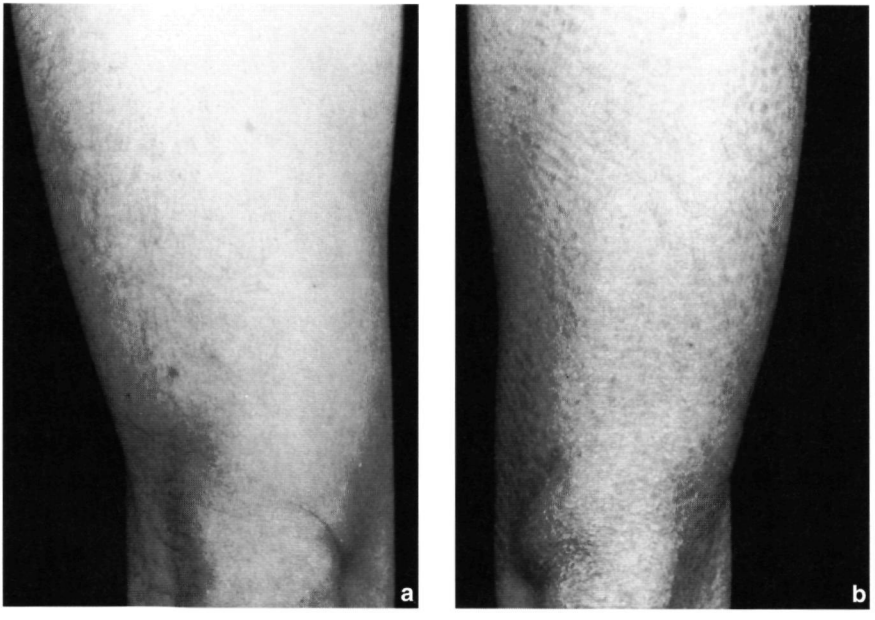

Fig. 2 a. The same patient as in Figure 1. After treatment, a significant unilateral improvement was observed in favour of the calcipotriol-treated right leg. **b.** Some improvement was also noted on the ointment base-treated left leg.

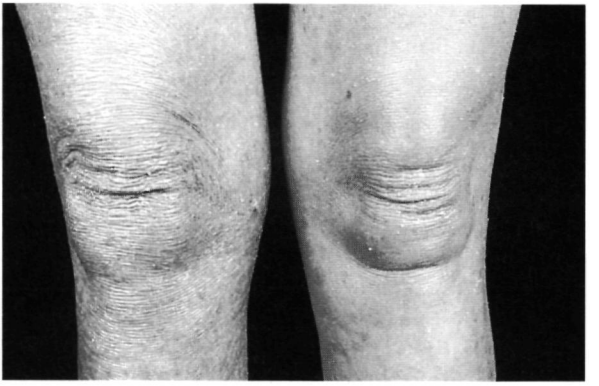

Fig. 3 EARLI patient after treatment. Substantial improvement is seen on the calcipotriol-treated left side. The scaling on the ointment base-treated right side consists of characteristic fine scales, and is comparable with the pretreatment state.

Topical 1α,25-dihydroxyvitamin D3 and systemic 1α-hydroxyvitamin D3 have been reported to be ineffective in the treatment of ADIV and XRI [10,15]. In these studies the concentration of 1α,25-dihydroxyvitamin D3 was 0.1 μg/g, and the oral dose of 1α-hydroxyvitamin D3 was 1 μg per day.

Calcipotriol is a safe and useful therapeutic agent, which exerts its effect by suppression of increased proliferation, and stimulation of terminal differentiation of the epidermis. Frost et al. described increased proliferative activity of the epidermis in lamellar and epidermolytic ichthyoses. ADIV and XRI, however, were characterized by normal proliferative activity [16,17]. Recent investigations, using flow cytometry, have confirmed these data. Analysis of the lamellar ichthyoses, which are a heterogeneous group, revealed a pronounced increase in proliferative activity in EARLI, whereas NEARLI exhibited only moderate epidermal hyperproliferation. Furthermore, the Comèl-Netherton syndrome showed also a high degree of hyperproliferation. It is attractive to speculate that these differences in epidermal cell proliferation rate might explain the different clinical responses to treatment with vitamin D3. The lack of improvement in ichthyosis bullosa of Siemens might be explained by the pronounced lichenified hyperkeratosis, which may prevent adequate penetration of calcipotriol.

We conclude that calcipotriol appears to be a useful new approach to the treatment of lamellar ichthyoses, bullous ichthyotic erythroderma of Brocq, and possibly the Comèl-Netherton syndrome. However, treatment of large areas of ichthyotic skin will require substantial amounts of calcipotriol ointment, and this constitutes a limiting factor in this approach to the therapy of ichthyoses. The potential risk of adverse biochemical effects could be lessened by using smaller quantities weekly, or by limiting the application frequency to three times a week. If quantities of calcipotriol > 120 g weekly are used, it

is essential to monitor serum calcium and 24-h urinary calcium excretion. The latter is a more sensitive parameter than the serum level [18].

Future studies are required in order to obtain additional information on the use of calcipotriol in the treatment of ichthyoses, and to assess the clinical efficacy of calcipotriol in the treatment of other hyperproliferative epidermal diseases.

ACKNOWLEDGEMENTS

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REFERENCES

1. Traupe H. The Sjögren-Larsson syndrome. In: The Ichthyoses. Springer Verlag Berlin 1989;157-162
2. Binderup L, Bramm E. Effects of a novel vitamin D analogue MC 903 on cell proliferation and differentiation in vitro and calcium metabolism in vivo. Biochem Pharmacol 1988;37:889-95
3. Clemens TL, Adams JS, Horiuchi N et al. Interaction of 1,25-dihydroxyvitamin D3 with keratinocytes and fibroblasts from skin of normal subjects and a subject with vitamin D dependent rickets, type II: A model for study of mode of action of 1,25- dihydroxyvitamin D3. J Clin Endocrinal Metab 1983;56:824-30
4. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. J Am Acad Dermatol 1988;19:516-28
5. Smith EL, Walworth NC, Holick MF. Effect of 1,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum free conditions. J Invest Dermatol 1986;86:709-14
6. Kondo S, Satoh Y, Yamaguchi J. The inhibitory effects of 1,25-dihydroxyvitamin D3 on DNA synthesis in cultured epidermal cells from patients with psoriasis vulgaris. Nishi Hihon J Derm 1988;50:679
7. Kragballe K. MC 903, a non-calciotropic vitamin D3 analogue stimulates differentiation and inhibits proliferation of cultured human keratinocytes. J Invest Dermatol 1988;91:383
8. Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T. Regulation of terminal differentiation of culture mouse epidermal cells by 1,25-dihydroxyvitamin D3. Endocrinology 1983;3:1950-57
9. Holick MF, Smith E, Pincus S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxyvitamin D3. Arch Dermatol 1987;123:1677-1683
10. Okano M. 1,25-(OH)2D3 use on psoriasis and ichthyosis. Int J Dermatol 1991;30:62-64
11. Kragballe K. Treatment of psoriasis by the topical application of the novel vitamin D3 analogue MC 903. Arch Dermatol 1989;125:1647-52
12. Kragballe K, Gjertsen BT, de Hoop D et al. Double-blind, right-left comparison of calcipotriol and betamethasone valerate in treatment of psoriasis vulgaris. Lancet 1991;337:193-6.
13. Van de Kerkhof PCM, de Jong EMGJ. Topical treatment with the vitamin D3 analogue MC903 improves pityriasis rubra pilaris: clinical and immunohistochemical observations. Br J Dermatol 1991;125:293-294
14. Berth-Jones J, Hutchinson PE. Vitamin D analogues and psoriasis. Br J Dermatol 1992;127:71-78.

15. Okano M, Kitano Y, Yoshikawa K. A trial of oral 1-hydroxyvitamin D3 for ichthyosis. Dermatologica 1988;177:23
16. Frost P, Weinstein GD, van Scott EJ. The ichthyosiform dermatoses II. Autoradiographic studies of epidermal proliferation. J Invest Dermatol 1966;47:561-567.
17. Frost P. Ichthyosiform dermatoses. J Invest Dermatol 1973;60:541-552
18. Holick MF, Pochi P, Bhawan J. Topically applied and orally administered 1,25-dihydroxyvitamin D3 is a novel, safe, and effective therapy for the treatment of psoriasis: a three year experience with histologic analysis. J Invest Dermatol 1989;92:446.

Chapter 3

Topical treatment of Sjögren-Larsson syndrome with calcipotriol

GPH Lucker
PCM van de Kerkhof
JRM Cruysberg
DJ der Kinderen
PM Steijlen

Dermatol 1995;190:292-294

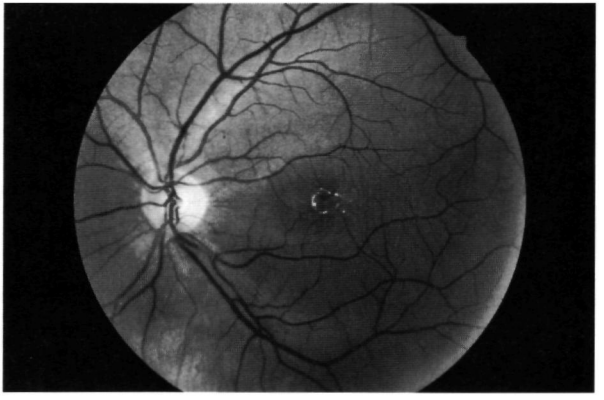
SUMMARY

Two patients with Sjögren-Larsson syndrome were treated with calcipotriol in ointment and the ointment base only for 12 weeks, using a double-blind bilaterally paired comparative study. Unilateral improvement was observed in both patients in favour of the calcipotriol-treated side. The present case is the first demonstration of a substantial clinical effect of calcipotriol in the Sjögren-Larsson syndrome.

INTRODUCTION

The Sjögren-Larsson syndrome (SLS) is a rare, autosomal recessively inherited disorder with an estimated incidence of 1:200.000. Characterizing features of this neuroectodermal genodermatosis are ichthyosis and neurologic manifestations, while a number of associated symptoms may be present. The outstanding clinical feature of ichthyosis in SLS is the congenital, yellowish to dark brown, keratotic lichenification of the skin. Signs of the neurologic manifestations, i.e. pathologic reflexes, muscular hypertonia, spastic di- or tetraplegia and mental retardation, usually emerge between 4 and 30 months of age and are stationary after puberty [1]. High-field magnetic resonance imaging may reveal atrophic changes of the cerebral motor area, extrapyramidal system, corpus callosum and spinal cord [2]. SLS may be associated with a variety of other anomalies. The presence of glistening dots on the macular region of the retina is regarded as pathognomonic, although not present in all patients. These dots are due to focal microglia degeneration and lead to impaired central vision [3,4]. Amblyopia may further reduce visual acuity [5]. Hyperkeratosis of palms and soles as well as joint hyperextensibility have been reported in association with the SLS [6]. Biochemically, a deficiency of the fatty aldehyde dehydrogenase component [7] of the fatty alcohol: NAD+ oxidoreductase complex has been established in cultured fibroblasts and leucocytes [8.9], while a partial reduction has been reported in obligate carriers [10]. Using a histochemical technique, a deficiency of fatty alcohol: NAD+ oxidoreductase activity in epidermis and jejunal mucosa has been demonstrated [11].

This enzyme deficiency may constitute the biochemical link between the skin and neurologic symptoms, for fatty alcohol: NAD+ oxidoreductase deficiency causes accumulation of fatty alcohols in keratinocytes and neurons. The fatty alcohols are subsequently incorporated into lipids essential for normal cell membrane functions. Recently, however, 2 patients with the typical clinical features of SLS have been reported with normal fatty alcohol: NAD+ oxidoreductase activity, possibly suggesting that SLS is a heterogeneous disease [12,13]. Vitamin D3 (dihydroxycholecalciferol) causes a dose-dependent decrease in proliferation and an increase in differentiation of human cultured keratinocytes [14]. Clinical data indicate that externally applied vitamin D3 is effective in alleviating psoriasis, where proliferative activity of the epidermis is greatly increased [15]. This study was part of a large multicentre study [16] and was intended to be an illustration of the effect of calcipotriol in SLS.



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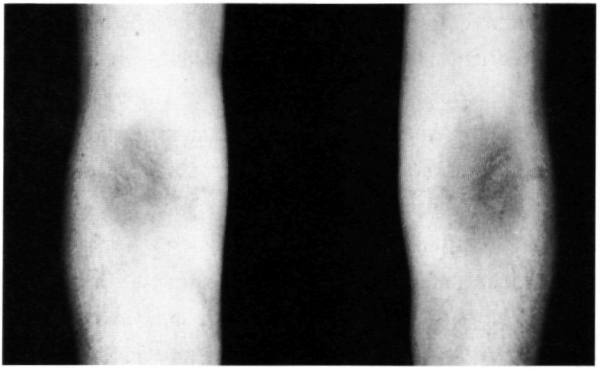

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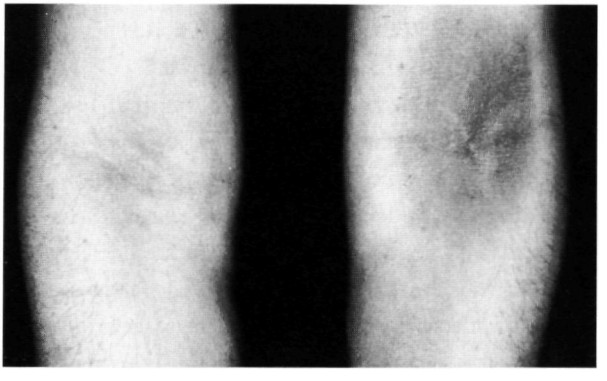

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Fig. 1 Pathognomonic glistening dots in the macular region of patient I.
Fig. 2 Patient II. Both elbows equally covered with ichthyosis before treatment.
Fig. 3 Same patient as in figure 2. Results after 12 weeks of treatment. The ichthyosis at the calcipotriol treated right side has almost completely disappeared. The ointment base treated left side is still covered with ichthyosis similar to the pretreatment observations.

PATIENTS AND METHODS

Patients

Sionce the age of 10 months, a 35-year-old man had shown an ichthyosis with a yellow to brownish lichenified appearance, particularly pronounced on the flexural surfaces and on the lower abdomen. In childhood a spastic diplegia, impaired vision and photophobia became manifest. His 38-year-old sister had a similar skin condition, though less extensive, without neurologic or ophthalmologic complaints. Funduscopic examination revealed a brightened macular region of the retina in both patients. Pathognomonic small glistening dots were observed only in patient I (Fig. 1). Histologic findings consisted of orthokeratosis, focal parakeratosis, acanthosis and focal papillomatosis of the epidermis. To confirm the clinical diagnosis, a biochemical assay was carried out, measuring the fatty aldehyde dehydrogenase component of fatty alcohol:NAD+ oxidoreductase in cultured fibroblasts. The assay was carried out as described by Rizzo and Craft [7] using hexadecanal as substrate. Fatty aldehyde dehydrogenase values were respectively 496 and 1486 pmol/min/mg protein (normal range 3320-4850) in patients I and II, respectively, and confirmed the clinical diagnosis.

Methods

The 2 patients were treated with topical calcipotriol after informed consent had been obtained. Topical (urea 10%) as well as systemic treatment (acitretin 10 mg daily) was stopped 2 and 10 weeks, respectively, before the treatment was started. To make sure that any effect of previous topical therapy disappeared, the patients only used an emollient (Locobase) as required, in the pretreatment wash-out period of 2 weeks. Using a double-blind, bilaterally paired comparative approach, the patients received calcipotriol ointment (50 μg/g) on one side of the body and the vehicle only on the opposite side. The ointments were applied thinly and evenly, twice daily, for a period of 12 weeks. A maximum amount of 120 g ointment per week was dispensed for each side of the body. The patients were instructed to wash their hands after application, to avoid inadvertent spread to other body areas. Blood samples for haematological and biochemical analysis were taken 2 weeks before the start of the study and at weeks 2 and 12 of the treatment phase. Clinical efficacy was established by measuring the extent of roughness, using a 4-point scale.

RESULTS

At the end of the study, a half-side reduction, in favour of the calcipotriol-treated side, was observed in both patients (Fig. 2, 3). No improvement was observed at the ointment-base-treated side. Apart from some discomfort at both sides due to the fatty base, no unilateral side- effects were reported. Blood parameters remained in the normal range during the study. In particular, there was no rise in serum calcium. Roughness appeared to be the best parameter to establish clinical improvement of the ichthyosis, characterized

by keratotic lichenification, in SLS. Erythema and scaling, however, were absent in both patients at the start of the study, and remained so till the study was completed.

DISCUSSION

This is the first demonstration of a therapeutic effect of topical calcipotriol in the SLS. In the present study, no clinical side-effects or laboratory abnormalities were found. Previous reports on topical treatment with 1α,25-dihydroxyvitamin D3 as well as systemic treatment with 1α-hydroxyvitamin D3 of autosomal dominant ichthyosis vulgaris (ADIV) and X-linked recessive ichthyosis (XRI), showed an ineffectiveness of vitamin D3 [17,18].

Frost [19] established hyperproliferation to be a contributing factor in the pathogenesis of lamellar ichthyosis and epidermolytic hyperkeratosis, but not in ADIV and XRI, using autoradiographic techniques with tritiated thymidine which is incorporated into cells in the period of DNA synthesis. Jagell and Liden [20] demonstrated the DNA synthesis of epidermal cells in SLS to be 2.7 times greater than normal, using the same technique. Therefore, the ichthyosis in SLS is characterized by hyperproliferation, whereas ADIV and XRI represent normoproliferative ichthyoses, possibly explaining the differences in clinical efficacy of the treatment with vitamin D3 analogues.

Apart from symptomatic therapies improving skin manifestations, a substitutional treatment has been proposed, in order to correct the basic lipid disorder underlying both dermatologic and neurologic symptoms [21,22].

An interesting speculation is the possible amelioration of the neurologic symptoms by supplementation of dopamine agonists, since severely reduced dopamine concentrations have been found in the putamen and, to a lesser extent, in the substantia nigra and other striatal regions, suggesting a specific mono-aminergic dysfunction in patients with SLS [23].

Further research remains to be done, in order to offer the patient with SLS a substitutional treatment, ameliorating both skin and neurologic symptoms. At the moment though, symptomatic treatment of the skin manifestations remains important. Topical calcipotriol constitutes a new and promising approach in alleviating the ichthyosis in SLS.

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REFERENCES

1. Traupe H. The Sjögren-Larsson syndrome. In: The Ichthyoses. Springer Verlag Berlin 1989;157-162
2. Morishita S, Konagaya M, Konagaya Y, Tahayanagi T. MRI study of three siblings of suspicious Sjögren-Larsson syndrome. Rinsho-Shinheigaku 1990;30:1118-22.
3. Daicker B. Zur Kenntnis von Substrat und Bedeutung der sogenannten Schneckenspuren der Retina. Ophthalmologica 1972;165:360-5.
4. Jagell S, Polland W, Sandgren O. Specific changes in the fundus typical for the Sjögren-Larsson syndrome. An ophthalmologic study. Acta Ophthalmol 1980;58:321-30.
5. Barnard NA, Patel C, Barnard RA. Sjögren-Larsson syndrome: case reports of two brothers. Ophthalmic Physiol Opt 1991;11:180-3.
6. Levisohn D, Dintiman B, Rizzo WB. Sjögren-Larsson syndrome: case reports. Pediatr Dermatol 1991;8:217-20.
7. Rizzo WB, Craft DA. Sjögren-Larsson syndrome. Deficient activity of the fatty aldehyde dehydrogenase component of fatty alcohol: NAD+oxidoreductase in cultured fibroblasts. J Clin Invest 1991;88:1643-8.
8. Rizzo WB, Dammann AL, Craft DA. Sjögren-Larsson syndrome: impaired fatty alcohol oxidation in cultured fibroblasts due to deficient fatty alcohol: nicotinamide adenine dinucleotide oxidoreductase activity. J Clin Invest 1988;81:738-44.
9. Rizzo WB, Dammann AL, Craft DA, Black SH, Tilton AH, Africk DCC, Holmgren G, Jagell S. Sjögren-Larsson syndrome inherited defect in the fatty alcohol cycle. J Pediat 1989;115:228-34.
10. Kelson TL, Craft DA, Rizzo WB. Carrier detection for Sjögren-Larsson syndrome. J Inherit Metab Dis 1992;15:105-11.
11. Judge MR, Lake BD, Smith VV, Besley GTN, Harper JI. Depletion of alcohol (hexanol) dehydrogenase activity in the epidermis and jejunal mucosa in Sjögren-Larsson syndrome. J Invest Dermatol 1990;95:632-4.
12. Koone MD, Rizzo WB, Elias PM. Ichthyosis, mental retardation and a symptomatic spasticity. A new neurocutaneous syndrome with normal fatty alcohol: NAD+ oxidoreductase activity. Arch Dermatol 1990;126:1485-90.
13. Yasaka N, Fujimoto M, Tamaki K. Detection of hexanol dehydrogenase activity in the epidermis of a patient with Sjögren-Larsson syndrome. Dermatol 1992;19:258-9.
14. Smith EL, Walworth NC, Holick MF. Effect of 1,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. J Invest Dermatol 1986;86:709-14.
15. Kragballe K. Treatment of psoriasis by the topical application of the novel vitamin D3 analogue MC 903. Arch Dermatol 1989;125:1647-52.
16. Kragballe K, Steijlen PM, Ibsen HH, et al. Efficacy, tolerability, and safety of calcipotriol ointment in disorders of keratinization: Results of a randomized, double-blind, vehicle-controlled, right-left comparative study. Arch Dermatol, in press.
17. Okano M. 1,25-(OH)2D3 on psoriasis and ichthyosis. Int J Dermatol 1991;30:62-4.
18. Okano M, Kitano Y, Yoshikawa K. A trial of oral 1-hydroxyvitamin D3 for ichthyosis. Dermatologica 1988;177:23.
19. Frost P. Ichthyosiform dermatoses. J Invest Dermatol 1973;60:541-552.
20. Jagell S, Liden S. Ichthyosis in the Sjögren-Larsson syndrome. Clin Genet 1982;21:243.

21. Guilleminault C, Harpey JP, Lafourcade J. Sjögren-Larsson syndrome. Report of two cases in twins. Neurology 1973;23:367-373.
22. Hooft C, Kriekemans J, van Acker K, Devos E, Traen S, Verdonck G. Sjögren-Larsson syndrome with exudative enteropathy. Influence of medium-chain triglycerides on the symptomatology. Helv Paediatr Acta 1967;5:447-58.
23. Wester P, Bergstrom U, Brun A, Jagell S, Karlsson B, Eriksson A. Monoaminergic dysfunction in Sjögren-Larsson syndrome. Mol Chem Neuropathol 1991;15:13-28.

Chapter 4

Flow-cytometric investigation of epidermal cell characteristics in monogenic disorders of keratinization and their modulation by topical calcipotriol treatment

GPH Lucker
PM Steijlen
EJA Suykerbuyk
K Kragballe
F Brandrup
PCM van de Kerkhof

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SUMMARY

A flow-cytometric study was performed in monogenic disorders of keratinization, to assess DNA distribution as well as the expression of keratins and involucrin. In addition, the changes in expression of these markers under influence of calcipotriol treatment were investigated.

Proliferation, measured by the percentage of epidermal cells in SG2M- phase of the cell cycle, was increased in Darier's disease, lamellar ichthyosis, congenital bullous ichthyotic erythroderma of Brocq and the Comel-Netherton syndrome, whereas normal proliferation was found in autosomal dominant ichthyosis vulgaris, X-linked recessive ichthyosis, keratosis pilaris, ichthyosis bullosa of Siemens and the Sjögren-Larsson syndrome. Keratin 6 was enhanced in erythrodermic lamellar ichthyosis, congenital bullous ichthyotic erythroderma of Brocq and the Comel-Netherton syndrome, showing also reduction of keratin 10. Involucrin was only slightly reduced in erythrodermic lamellar ichthyosis, congenital bullous ichthyotic erythroderma of Brocq and the Comel-Netherton syndrome, compared to the pronounced reduction in all other skin disorders studied.

Calcipotriol was found to enhance differentiation in Darier's disease, erythrodermic lamellar ichthyosis, and congenital bullous ichthyotic erythroderma of Brocq. Only Darier's disease did not show clinical improvement.

In conclusion, flow cytometry provides a useful method for quantification of epidermal cell characteristics in monogenic disorders of keratinization. Further studies need to be performed to establish its usefulness as a diagnostic and prognostic tool.

INTRODUCTION

One of the changes of normal, terminally differentiating keratinocytes is the specific expression of keratin intermediate filaments. Several methods for detection of these intermediate filament proteins are available. Two-dimensional polyacrylamide gel electrophoresis is most often used to characterize keratin proteins biochemically (1,2). This method allows specific detection of the whole repertoire of keratins present in a tissue specimen, though the localization of the keratins is lost. Immunohistochemical methods using monoclonal antibodies recognizing single or sets of related keratins have the advantage that topographical information is maintained (3-5). A limitation of this approach is that only qualitative or semi-quantitative information can be obtained. Flow-cytometric analysis of intermediate filament proteins in single cell suspensions of epidermal cells allows rapid simultaneous quantification of two or more characteristics per individual cell, with high reproducibility and statistical accuracy (6). Immunohistochemical and flow-cytometric approaches have been used to determine the epidermal cell characteristics in normal and psoriatic skin and to evaluate the effects of therapies on proliferation and differentiation in psoriasis (7-9). Little information is available on epidermal cell kinetics in monogenic disorders of keratinization. Some immunohistochemical studies have been performed with the monoclonal antibody Ki-67,

recognizing a proliferation-associated nuclear antigen (7). However, no quantitative data are available on epidermal cell characteristics in monogenic disorders of keratinization, using a flow-cytometric approach.

The vitamin D3 analogues calcipotriol and calcitriol (1α25-dihydroxyvitamin D3) have been shown to inhibit cell proliferation (10-12) and induce terminal differentiation (10,13-15) in cultured human keratinocytes. Immunohistochemical evaluation of treatment of psoriatic plaques with calcipotriol has revealed a rapid decrease of the number of proliferating cells, measured as Ki-67-positive nuclei, after 2 weeks, resulting in a total decrease of 48% after 12 weeks. Ks8.12-binding revealing keratin 16 expression has shown a tendency to diminish after 4-12 weeks of treatment (8). Flow-cytometric analysis has shown a decrease in DNA-content and a reduction of Ks8.12-binding in psoriasis, after 6 weeks of treatment with calcipotriol (9).

The aim of the present investigation was to assess, using a flow-cytometric approach, DNA distribution, as well as the expression of intermediate filament proteins and involucrin, markers for epidermal proliferation and differentiation, in order to characterize epidermal cell characteristics in Darier's disease, keratosis pilaris (KP), autosomal dominant ichthyosis vulgaris (ADIV), X-linked recessive ichthyosis (XRI), and congenital ichthyoses. Furthermore, the changes in expression of these keratins under influence of calcipotriol treatment were analyzed using flow cytometry.

PATIENTS AND METHODS

Patients

Twenty-five patients (3 ADIV, 5 XRI, 5 Darier, 3 KP, 9 congenital ichthyosis) participated in the study. The group of congenital ichthyoses consisted of 1 erythrodermic lamellar ichthyosis (ELI), 2 non-erythrodermic lamellar ichthyosis (NELI), 1 ichthyosis bullosa of Siemens (Siemens), 2 congenital bullous ichthyotic erythroderma of Brocq (Brocq), 1 Comel-Netherton syndrome (CNS), and 2 patients with the Sjögren-Larsson syndrome (SLS). A superficial skin sample, about 0.2 mm thick and 3 mm in diameter, was removed from a representative area from each side with a razor blade in conjunction with a metal guard, from all patients before treatment. Similar samples were taken from eleven healthy volunteers. Eighteen patients (2 ADIV, 5 XRI, 3 Darier, 3 KP, 1 ELI, 2 NELI, 1 Siemens and 1 Brocq) completed a double-blind bilaterally paired comparative treatment period of 12 weeks with calcipotriol ointment 50 μg/g and the ointment vehicle only. At week 12, similar biopsies were taken from both sides. All 25 patients were recruited from the centre in Nijmegen. Twenty-four of them (except for one ADIV) constituted a subgroup, participating in a multicenter study from which the clinical results have been published before (16). For practical reasons only skin samples obtained from patients treated at the centre of dermatology in Nijmegen were analyzed using flow cytometry.

Monoclonal antibodies

- AF124 (National Cancer Institute, Bethesda, Maryland, USA)(17) to assess keratin 6, which is expressed in hyperproliferative epidermis.
- RKSE60 (Department of Pathology, University Hospital Maastricht, Netherlands)(18) to assess keratin 10 expression, present in differentiated epidermis.
- Anti- involucrin (Biomedical Technologies, Inc, Stoughton, USA)(19) to assess involucrin expression, a marker for terminal differentiation.
- 6B10 (Eurodiagnostics, Apeldoorn, Netherlands)(20) to assess keratin 4, which is expressed following treatment with retinoids.
- 1C7 (Eurodiagnostics, Apeldoorn, Netherlands)(20) to assess keratin 13, which is also expressed following retinoid treatment.

Analytical procedures

Cell suspensions were prepared using a trypsinization procedure in order to dissociate biopsy specimens into single cells. The biopsy specimen was washed in phosphate-buffered saline (PBS) and incubated with the dermal site downward in 1 ml of PBS containing 1% trypsin (DIFCO, Detroit, Mich.) and 0.3% dithioerythritol (Sigma, St. Louis, Mo.) at 37°C for 20 min. Subsequently, the specimen was fixed in 500 µl ice-cold 70% ethanol and dissociated by sonification (Sonifier B-12, Branson Sonic Power, Danbury, Coun.) for 5 s at 70W. This resulted in a cell suspension containing only epidermal cells, for dermis and stratum corneum remained intact and floated on the surface.

Aliquots of these ethanol-fixed cell suspensions were immunostained with the monoclonal antibodies anti-involucrin, 6B10, 1C7, RKSE60 and AF124. As second antibody, goat anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) (GAM-FITC; Tago, Burlingame, U.S.A.) was used for all stainings, except for detection of AF124 which required a FITC-conjugated swine anti-rabbit IgG (SAR-FITC; Dakopatts, Copenhagen, Denmark). These antibodies were added in a 1:25 dilution for 30 min at room temperature. After the two incubation steps, the cells were washed twice and resuspended in 200 µl PBS, pH 7.8, containing propidium iodide (PI) 40 mg/l (Calbiochem, San Diego, U.S.A.). Afterwards the cell suspensions were incubated for 15 min with 50µl RNAse (1mg/ml in PBS) (Sigma, St. Louis, U.S.A.) at room temperature and filtered in order to remove clumps; fluorescence was measured using an Ortho 50H flow cytometer (Ortho Instruments, Westwood, U.S.A.). Both FITC and PI were excited with a 5W argon laser (164-05, Spectra Physics, Mountain View, U.S.A.) tuned at 488 nm, and emission was recorded at 515-530 nm (FITC) and >630 nm (PI). The data were stored and analyzed with a Digital PDP 11/34 computer (Digital Equipment, Galway, Republic of Ireland). Green fluorescence derived from FITC indicated staining with the primary antibodies. Red fluorescence derived from PI was used to establish the DNA content per cell, allowing a differentiation between the cells in the consecutive phases off

the cell cycle. The cells with elevated DNA content reflected the cells in the SG2M phase of the cell cycle. Therefore, the percentage of cells in SG2M phase was measured as a marker of proliferation.

RESULTS

Epidermal cell characteristics in monogenic disorders of keratinization

Flow-cytometric measurements of cell suspensions obtained in the various monogenic disorders of keratinization are summarized in Table I.

The SG2M values have been measured for each of the monoclonal antibodies, resulting in a total amount of five SG2M values in each sample. In the tables, the mean values are shown ± S.E.M. As most disorders are extremely rare, the number of patients in the various groups was too low to perform statistical analysis. To allow a comparison between the flow-cytometric data before calcipotriol treatment found in the various skin disorders, alterations more than one standard deviation from the mean values in the normal control group were taken into consideration.

The percentage of cells in the SG2M phase of the cell cycle was elevated in Darier, ELI, NELI, Brocq, and CNS. Values comparative to normal were observed in ADIV, XRI, KP, Siemens, and the SLS. Keratin 6 revealed an increase in ELI, Brocq, and CNS. A reduction of keratin 10 was observed in CNS. A reduction, less than one standard deviation from the normal controls, was noticed in Darier, ELI, NELI, Siemens, and SLS. Involucrin was diminished in all monogenic disorders of keratinization studied. ELI, Brocq, and CNS exhibited only a reduction of involucrin expression of more than one standard deviation, whereas a reduction of more than three standard deviations was observed in the other dermatoses. The percentage of cells containing keratins 4 and 13 was low in all subgroups.

Modulation of epidermal cell characteristics by calcipotriol treatment

To allow a comparison between the flow-cytometric changes by calcipotriol treatment, alterations of the nett calcipotriol effect of more than one standard deviation from the mean pretreatment values were taken into consideration.

Changes in epidermal cell characteristics in the monogenic disorders of keratinization, after treatment with calcipotriol, compared to its vehicle, are summarized in Table II.

An increase in involucrin expression in favour of the calcipotriol treated side was observed in Darier, ELI, and Brocq.

Alteration of keratin 10 expression paralleled the changes observed in involucrin expression. Again, a unilateral increase in favour of the calcipotriol treated side was observed exclusively in Darier, ELI and Brocq. Keratin 6 expression in ADIV, KP and Siemens was reduced by calcipotriol, while an elevation was noticed in Darier, ELI and Brocq.

Table I. Mean values (± SEM) for percentage of cells positive for markers of proliferation and differentiation in normal skin and monogenic disorders of keratinization

	Normal n=11	ADIV n=3	XRI n=5	Darier n=5	KP n=3	ELI n=1	NELI n=2	Siemens n=1	Brocq n=2	CNS n=1	SLS n=2
Involucrin	45.2±2.7	18.1[c]±4.5	12.8[c]±1.6	18.3[c]±1.9	17.5[c]±2.8	35.0[a]±0.1	4.0[c]±1.3	10.1[c]±2.1	29.4[a]±1.5	29.7[a]±0.1	9.5[c]±1.6
Keratin 4	0.9±0.2	2.7[b]±0.9	3.9[c]±1.3	5.5[c]±1.1	2.9[b]±0.8	1.5±0.0	1.7[a]±0.5	4.0[c]±0.7	2.6[b]±0.4	2.1[a]±0.1	0.8±0.0
Keratin 13	3.8±0.5	2.2±0.6	3.8±1.2	5.7[c]±1.4	3.2±0.8	1.6±0.0	1.3±0.2	3.0±0.5	2.0±0.3	1.7±0.3	0.6±0.0
Keratin 10	55.4±5.3	57.6±4.0	56.1±2.9	48.4±4.1	59.7±4.1	45.0±1.2	51.9±1.4	50.4±5.5	57.3±4.6	36.5[a]±2.6	52.7±4.1
Keratin 6	18.8±2.9	9.9±4.8	5.3±1.0	16.0±1.7	7.7±0.9	54.2[c]±1.6	19.5±7.1	2.3±0.7	35.8[a]±4.9	47.1[b]±4.9	3.0±0.5
SG2M	5.8±0.8	6.5±0.3	7.3±0.4	10.7[a]±0.6	6.5±0.2	11.1[a]±0.4	8.5[a]±0.4	8.1±0.8	10.6[a]±0.4	9.1[a]±0.6	5.8±0.4

[a]>1 SD from normal [b]>2 SD from normal [c]> 3 SD from normal

Table II. Changes (± S.E.M.) of epidermal cell characteristics in monogenic disorders of keratinization under influence of calcipotriol treatment

	ADIV (n=2)	XRI (n=5)	Darier (n=3)	KP (n=3)	ELI (n=1)	NELI (n=2)	Siemens (n=1)	Brocq (n=1)
Involucrin calcipotriol	7.1 ±4.9	-0.2 ±0.8	12.6 ±4.4	12.0 ±6.7	9.6	0.2 ±0.0	6.8	25.6
vehicle	7.9 ±5.7	2.1 ±1.0	-0.9 ±3.2	10.0 ±5.8	-13.5	0.6 ±0.7	15.4	7.9
difference	-0.8 ±0.8[c]	-2.3 ±1.0	13.5 ±7.5[c]	2.0 ±10.3	23.1[c]	-0.4 ±0.7	-8.6[c]	17.7[a]
Keratin 4 calcipotriol	1.6 ±0.4	-2.3 ±1.7	7.2 ±1.7	-0.3 ±1.0	0.9	-0.2 ±3.5	0.5	0.5
vehicle	-1.4 ±1.8	-1.3 ±1.7	-0.4 ±1.4	1.5 ±2.3	0.7	-1.2 ±0.6	2.6	-0.2
difference	3.0 ±2.2[b]	-1.0 ±2.9	7.6 ±2.4[c]	-1.8 ±3.2[a]	0.2[c]	1.0 ±0.4[a]	-2.1[c]	0.7[a]
Keratin 13 calcipotriol	1.2 ±0.1	-3.0 ±1.7	5.5 ±3.0	0.2 ±0.5	1.2	-0.5 ±0.1	0.3	0.4
vehicle	0.2 ±0.6	0.3 ±0.1	0.3 ±0.9	1.8 ±1.0	2.2	0.0 ±0.0	-0.2	0.1
difference	1.0 ±0.5[a]	-3.3 ±1.7[a]	5.2 ±3.4[a]	-1.6 ±1.4[a]	-1.0[c]	-0.5 ±0.1[a]	0.5[a]	0.3
Keratin 10 calcipotriol	-2.1 ±4.8	-4.6 ±2.0	3.1 ±3.8	2.1 ±2.2	-3.4	-1.1 ±1.1	-2.2	20.4
vehicle	0.6 ±2.4	-4.8 ±3.2	-7.5 ±14.5	2.7 ±5.2	-5.7	1.2 ±0.1	5.4	7.0
difference	-2.7 ±2.5	0.2 ±2.4	10.6 ±13.5[a]	-0.6 ±3.3	2.3[a]	-2.3 ±1.1[a]	-7.6[a]	13.4[b]
Keratin 6 calcipotriol	-6.0 ±4.5	0.5 ±1.8	10.6 ±14.3	4.8 ±4.0	18.1	-7.2 ±5.1	0.0	28.9
vehicle	7.7 ±5.4	-1.0 ±2.8	5.3 ±10.5	7.6 ±2.2	9.2	-12.0 ±4.1	11.8	6.9
difference	-13.7 ±10.0[a]	1.5 ±3.5	5.3 ±4.3[a]	-2.8 ±6.1[a]	8.9[c]	4.8 ±1.0	-11.8[c]	22.0[c]
SG2M calcipotriol	0.6 ±0.2	0.2 ±0.4	-2.0 ±0.9	0.3 ±0.2	4.5 ±1.1	1.5 ±0.4	-4.3 ±1.3	0.5 ±2.4
vehicle	-0.1 ±0.4	0.9 ±0.5	-1.5 ±0.2	0.7 ±0.2	3.5 ±1.9	-0.6 ±0.3	2.3 ±2.0	1.5 ±1.0
difference	0.7 ±0.0[a]	-0.7 ±0.8	-0.5 ±1.0	-0.4 ±0.2[a]	1.0 ±1.9[b]	2.1 ±0.4[c]	-6.6 ±2.1[c]	-1.0 ±1.5[a]

The values indicated in the Table represent the changes in mean values of percentage of cells positive for the monoclonal antibodies from baseline to end of treatment. The changes are indicated for the calcipotriol- and verum-treated side. The nett calcipotriol effect is indicated by the difference.
[a]>1 SD from normal [b]>2 SD from normal [c]>3 SD from normal

Modulation of keratins 4 and 13 was rather inconsistent: keratins 4 and 13 were both upregulated by calcipotriol in ADIV and Darier. In KP both were downregulated. Contradictory modification was observed in ELI, NELI, and Siemens. In Brocq only keratin 4 was modified upward, whereas in XRI only keratin 13 was regulated downward.

Only moderate modulation was observed in the percentage of cells in SG2M phase. An increase was observed in ADIV, ELI and NELI, whereas a decrease was found in KP, Siemens and Brocq.

DISCUSSION

In the present study, information on epidermal cell kinetics of monogenic disorders of keratinization was obtained, using a flow-cytometric approach. Furthermore, the influence of calcipotriol on proliferation and differentiation was analysed using flow cytometry.

Proliferation, measured by the percentage of cells in SG2M phase of the cell cycle, revealed a hyperproliferative state of the epidermis in Darier, ELI, NELI, Brocq and CNS, whereas proliferation was comparative to normal in ADIV, XRI, KP, Siemens and SLS. In 1966, Frost et al. (21) established hyperproliferation to be a contributing factor in the pathogenesis of lamellar ichthyosis, epidermolytic hyperkeratosis and Darier's disease, but not in ADIV and XRI, using autoradiographic technics with tritiated thymidine which is incorporated into cells in the period of DNA synthesis (22). Jagell and Liden demonstrated DNA synthesis of epidermal cells in SLS to be 2.7 times greater than normal, using the same technique (23). Our flow-cytometric data are in accordance with these previous observations, except for Siemens and SLS. This discrepancy may be explained by the fact that both Siemens and SLS are clinically characterized by prominent lichenification, implicating that relatively more superficial than basal epidermal cell layers may be obtained, using a 0.2 mm thick razor blade. Another marker for proliferation, AF124 staining keratin 6, was elevated in ELI, Brocq and CNS. Information on differentiation was obtained by the percentage of cells, positive for keratin 10, which is a marker for early differentiating keratinocytes. Keratin 10 was reduced more than one standard deviation compared to the normal controls only in CNS. However, all skin disorders characterized by hyperproliferation (as demonstrated with the SG2M values described previously) showed a reduced staining pattern for keratin 10, with the exception of Brocq, which is another ichthyosis subtype, clinically characterized by prominent lichenification. These data are in accordance with clonal analysis, growth kinetics, and cell cycle studies of normal human keratinocytes cultured in serum-free medium, which show that if keratinocyte proliferation is promoted, differentiation is inhibited (24).

Involucrin is one of the substrates for the formation of cornified envelopes and is synthesized in normal epidermis in the granular cell layer, implying that this protein is a

marker of late-stage differentiation. However, in hyperproliferative skin disorders such as benign neoplasms, malignant tumours, psoriasis, and DLE, involucrin is synthesized much earlier (25,26). Our study demonstrated diminished involucrin expression in all skin disorders studied. However, ELI, Brocq and CNS, all characterized by elevated keratin 6 expression and hyperproliferation, exhibited a more pronounced staining of anti-involucrin, compared to the other dermatoses studied.

Concerning the influence of calcipotriol on epidermal cell kinetics, we observed induction of differentiation, indicated by keratin 10 expression, as well as induction of terminal differentiation, indicated by involucrin expression, in Darier, ELI and Brocq, which is in accordance with previous studies on calcipotriol in psoriasis (27). Nevertheless, the expected reduction in proliferation, as was observed previously in psoriasis, was lacking in our study.

Keratins 4 and 13, which are expressed following treatment with retinoids (28), are normally found in fetal, but not in adult epidermis. Modulation after treatment with calcipotriol revealed no consistent findings.

Comparing the flow-cytometric data with the clinical data revealed a good correlation in KP and Siemens, which were unresponsive to calcipotriol, and in ELI and Brocq, which improved substantially. This in contrast to NELI, XRI and ADIV, in which some clinical improvement was observed, whereas the flow cytometry remained unchanged. Finally, it is of interest that Darier was characterized by flow-cytometric changes, although clinical improvement was not observed. It is attractive to speculate that Darier's disease might be responsive to calcipotriol when used at a lower and less irritating concentration.

We conclude that flow cytometry provides a useful method for establishing epidermal cell kinetics of monogenic disorders of keratinization. Further studies are required to find out to what extent quantification of cell cycle kinetics and markers of keratinization is useful as a diagnostic and prognostic tool.

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REFERENCES

1. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: Patterns of expression of specific cytokeratins in normal epithelia, tumours and cultured cells. Cell 1982;31:11-24.
2. Moll R, Moll I, Franke WW. Differences of expression of cytokeratin polypeptides in various epithelial skin tumours. Arch Dermatol Res 1984;276:349-363.
3. Woodcock-Mitchell J, Eichner R, Nelson WG, Sun TT. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. J Cell Biol 1982;95:580-588.

4. Huszar M, Gigi-Leitner O, Moll R, Franke WW, Geiger B. Monoclonal antibodies to various acidic (type 1) cytokeratins of stratified epithelia. Selective markers for stratification and squamous cell carcinomas. Differentiation 1986;31:141-153.
5. Ramaekers FCSM, Puts JJG, Moesker O. Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumours: An overview. Histochem J 1983;15:691-713.
6. Erp PEJ van, Rijzewijk JJ, Boezeman JBM, Leenders J, Mare S de, Kerkhof PCM van de, et al. Flow cytometric analysis of epidermal subpopulations from normal and psoriatic skin using monoclonal antibodies against intermediate filaments. Am J Pathol 1989;135:865-870.
7. Jong EMG de, Kerkhof PCM van de. Simultaneous assessment of inflammation and epidermal proliferation in psoriatic plaques during long-term treatment with vitamin D3 analogue MC 903: Modulations and interrelations. Br J Dermatol 1991;124:221-229.
8. Mare S de, Erp PEJ van, Jong EMGJ de, Kerkhof PCM van de. DNA content and Ks8.12 binding of the psoriatic lesion during treatments with the vitamin D3 analogue MC 903 and betamethasone. Br J Dermatol 1990;123:291-296.
9. Erp PEJ van, Groenendal H, Bauer FW, Rijzewijk JJ. Growth fraction in epidermal skin disorders determined by the monoclonal antibody Ki-67. J Invest Dermatol 1987;89:313.
10. Kragballe K. MC 903, a non-calciotropic vitamin D3 analogue stimulates differentiation and inhibits proliferation of cultured human keratinocytes. J Invest Dermatol 1988;91:383.
11. Smith EL, Walworth NC, Holick MF. Effect of 1,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum free conditions. J Invest Dermatol 1986;86:709-14.
12. Kondo S, Satoh Y, Yamaguchi J. The inhibitory effects of 1,25-dihydroxyvitamin D3 on DNA synthesis in cultured epidermal cells from patients with psoriasis vulgaris. Nishi Hihon J Derm 1988;50:679.
13. Clemens TL, Adams JS, Horiuchi N. Interaction of 1,25-dihydroxyvitamin D3 with keratinocytes and fibroblasts from skin of normal subjects and a subject with vitamin D dependent rickets, type II: A model for study of mode of action of 1,25- dihydroxyvitamin D3. J Clin Endocrinal Metab 1983;56:824-830.
14. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. J Am Acad Dermatol 1988;193:516-528.
15. Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T. Regulation of terminal differentiation of culture mouse epidermal cells by 1,25-dihydroxyvitamin D3. Endocrinology 1983;3:1950-1957.
16. Kragballe K, Steijlen PM, Ibsen HH, Kerkhof PCM van de, Esmann J, Sorensen LH, et al. Efficacy, tolerability and safety of calcipotriol ointment in disorders of keratinization: results of a randomized, double-blind, vehicle-controlled, right-left comparative study. Arch Dermatol 1995;131:556-560.
17. Roop DR, Cheng CK, Titterington L, Meyers CA, Stanley JR, Steinert PM, et al. Synthetic peptides corresponding to keratin subunits elicit highly specific antibodies. J Biol Cem 1984;259:8037-8040.
18. Ramaekers FCS, Puts JJG, Moesker O, Kant A, Huijsmans A, Haag D, et al. Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors: an overview. Histochem J 1983;15:691-713.
19. Rice RH, Green H. Presence in human epidermal cells of a soluble protein precursor of the cross-linked envelope: activation of the cross-linking by calcium ions. Cell 1979;18:681-694.

20. Muijen GNP van, Ruiter DJ, Franke WW, Achtstätter T, Haasnoot WHB, Ponec M, et al. Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins no's. 4 and 13. Exp Cell Res 1986;162:97-113.
21. Frost P, Weinstein GD, van Scott EJ. The ichthyosiform dermatoses II. Autoradiographic studies of epidermal proliferation. J Invest Dermatol 1966;47:561-567.
22. Frost P. Ichthyosiform dermatoses. J Invest Dermatol 1973;60:541-552.
23. Jagell S, Liden S. Ichthyosis in the Sjögren-Larsson syndrome. Clin Genet 1982;21:243.
24. Wille JJ, Pittelkow MR, Shipley GD, Scott RE. Integrated control of growth and differentiation of normal human prokeratinocytes cultured in serum-free medium: Clonal analyses, growth kinetics, and cycle cell studies. J Cell Physiol 1984;121:31-44.
25. Murphy GF, Flynn TC, Rice RH, Pinkus GS. Involucrin expression in normal and neoplastic human skin: a marker for keratinocyte differentiation. J Invest Dermatol 1984;82:453-457.
26. Jong EMGJ de, Erp PEJ van, Ruiter DJ, Kerkhof PCM van de. Immunohistochemical detection of proliferation and differentiation in discoid lupus erythematosus. J Am Acad Dermatol 1991;25:1032-1038.
27. Holland DB, Roberts SG, Russell A, Wood EJ, Cunliffe WJ. Changes in epidermal keratin levels during treatment of psoriasis with the topical vitamin D3 analogue MC 903. Br J Dermatol 1990;122:284.
28. Steijlen PM, Happle R, Muijen GNP van, Kerkhof PCM van de. Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. Dermatologica 1991;182:178-183.

Chapter 5

Oral treatment of ichthyoses by the cytochrome-P450 inhibitor liarozole.

GPH Lucker
AMC Heremans
PJ Boegheim
PCM van de Kerkhof
PM Steijlen

Br J Dermatol, accepted.

SUMMARY

Liarozole, a novel imidazole derivative, inhibits the cytochrome P450-dependent 4-hydroxylation of retinoic acid, resulting in increased tissue levels of retinoic acid. Twelve male patients with ichthyosis were given oral Liarozole 150 mg twice daily in an open study for twelve weeks. Immunohistochemical parameters of inflammation, epidermal proliferation and differentiation were assessed before and after treatment. Extent and severity of the skin lesions was markedly reduced in all patients. Clinical side effects were reminiscent of those with synthetic retinoids. No relevant changes were found in the haematological, urinary and biochemical parameters. Immunohistochemical assessment showed a statistically significant induction of keratin 4 after liarozole treatment in 10/12 patients. In 2 of these patients keratin 13 was induced.

This open study showed that oral liarozole treatment was efficacious and well tolerated in the treatment of different types of ichthyosis. The immunohistochemical results suggest a retinoid mechanism as mode of action.

INTRODUCTION

Retinoids (vitamin A-acid derivatives) are important compounds that control proliferation and differentiation of epithelial tissues in mammals [1-4]. Accordingly, systemic treatment with retinoids has yielded good results in the treatment of disorders of keratinization such as ichthyosis. However, their toxicity and teratogenicity limit their use to severely affected patients [5-8]. Therefore a search for new drugs with a broader toxic-therapeutic window is indicated. Liarozole, a novel imidazole derivative, inhibits the cytochrome P450-dependent 4-hydroxylation of retinoic acid which is the physiological breakdown of all-trans-retinoic acid. In rats, liarozole increased the concentration of retinoic acid in plasma and tissue [9]. Liarozole also inhibits several cytochrome P450-dependent steps in steroid biosynthesis - mainly the conversion of androgens to estrogens (aromatase), progestins to androgens (17-hydroxylase, 17 -20-lyase) and of 11-deoxycorticosterone to corticosterone (11-hydroxylase) [10]. Administration of 150 mg liarozole bidaily for 10 days to male volunteers showed neither reduction in cortisol levels, nor on the cortisol surge after ACTH stimulation [11]. Apart from a transient decrease of testosterone levels no changes of the levels of this hormone were observed. Furthermore, no abnormalities were observed in cortisol, cholesterol and testosterone levels in psoriasis patients, treated with doses ranging from 75 to 150 mg bidaily [12]. One open trial with liarozole 75 mg bid to 150 mg bid in severe plaque psoriasis showed a significant improvement in 77% of the patients [12]. Evidence is accumulating that liarozole will provide a broad efficacy-side effect window. However final conclusions await further comparative studies. The aim of the present study was to investigate the effectiveness and tolerability of oral liarozole in 12 patients with hereditary ichthyoses. Additionally to get insight in the in vivo action of liarozole on these disorders, immunohistochemical parameters of inflammation, epidermal proliferation and differentiation were assessed before and after treatment.

PATIENTS AND METHODS

In an open prospective study, 12 male patients suffering from various forms of ichthyosis were included: 5 patients with X- linked recessive ichthyosis (XRI), 4 patients with non erythrodermic lamellar ichthyosis (NELI), 1 patient with erythrodermic lamellar ichthyosis (ELI) and 2 patients with bullous congenital ichthyosiform erythroderma (BCIE). They were treated with oral liarozole (liarozole fumarate) 150 mg twice daily, for 12 weeks. Topical as well as systemical treatments were stopped respectively 2 and 4 weeks before the start of the study. Patients were allowed to use bland emollients during the study except on the days of the visits. Only male patients, age 18 to 70 years, were included. XRI-patients had a documented steroidsulphatase deficiency, and sun exposure had to be avoided. Patients were examined before treatment, at week 0, 1, 2, 4, 8, 12 during the treatment phase, and 4 and 8 weeks after discontinuation of therapy. The severity of involvement was rated using a 4 point scale for scaling, roughness, erythema, bullae and hyperpigmentation, as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Scaling and roughness were recorded in all subgroups, erythema and bullae in ELI and BCIE, and hyperpigmentation only in XRI. The extent of involvement was expressed as the percentage of affected skin on arms, trunk and legs. An overall response to treatment was quantified by both the investigator and the patient, on a five-point scale: 0 = deteriorated, 1 = unchanged, 2 = moderately improved, 3 = markedly improved, and 4 = cleared.

Haematological (haemoglobin, haematocrite, red blood count, white blood count, differentiation, platelet count), biochemical (Na, K, Cl, Ca, P, urea, kreatinin, albumin, protein, total bilirubin, cholesterol, triglycerides, alcalic phosphatase, ASAT, ALAT, GGT, glucose), and urinary analysis (specific gravity, pH, protein, glucose, urobilinogen, bilirubin, red blood count, white blood count) were performed at entry, and at each visit after initiation of the treatment. Statistical analysis was carried out using the Wilcoxon signed-rank test for paired data.

In all 12 patients, punch biopsies were taken from representative skin lesions before and after treatment. The antibodies used to assess epidermal proliferation, keratinization, and epidermal and dermal inflammation are summarized in table I. Immunohistochemical procedures were carried out as described previously[13].

RESULTS

Severity of skin involvement, assessed by the investigator, is presented in Figure 1. For the extent of involvement a comparative curve was observed. As expected, the extent and severity of the ichthyosis deteriorated during the wash-out phase and the severity even during the first week of treatment. Thereafter, a clear clinical improvement for both the extent and severity of the skin lesions was achieved. At the end of the treatment phase, an impressive clinical improvement was achieved in all patients (Figures 2,3). The extent of skin lesions was statistically significantly reduced for arms ($p<0.001$), trunk ($p=0.002$)

and legs ($p<0.001$). The degree of scaling and roughness, also significantly improved on the arms ($p<0.001$), trunk ($p=0.001$) and legs ($p=0.001$). As hyperpigmentation, erythema, and bullae were only assessed in a few patients (respectively 5, 1, and 2), no statistics were carried out. Nevertheless, changes in hyperpigmentation were comparable with changes in roughness and scaling. Erythema was not remarkably influenced. For overall response, both the investigator and 11 patients assessed the treatment as a marked improvement. One patient reported a moderate improvement. In 6 patients after discontinuation of the treatment, the extent and severity of the lesions gradually worsened to reach pretreatment values after 8 weeks. The other six patients already required treatment after 4 weeks. Eleven patients experienced adverse events. The most frequently reported clinical side effects were dry lips (10 patients), itching (6 patients) and dry eyes (3 patients). These side effects were mild, not requiring discontinuation of treatment in any patient. Of the two patients who mentioned hair loss, one improved during treatment and the other patient improved upon discontinuation of treatment. One patient who was inflicted with BCIE developed multiple large bullae on a dose of 150 mg twice daily but these disappeared upon reduction of the dose to 150 mg daily. One patient with XRI developed urticaria after 2 weeks of treatment. After a temporary stop of 2 weeks the lesions did not reappear and no relapse occurred after gradual readministration of liarozole, first 150 mg daily for 2 weeks, followed by the initial dose of 150 mg bidaily. Dryness of the nose, headache, gastric pain, increased skin tension, burning skin sensation, dizziness, enhanced sweating, candidiasis and undefined erythema were each observed in one occasion.

Haematological, biochemical and urinary parameters, measured at week 12, were not significantly influenced by liarozole, compared with the pretrial visit.

Table I. Specificity and sources of the antibodies.

Antibody	specificity	source
Proliferation		
Ki-67	nuclear antigen	Dakopatts, Copenhagen, DU
Keratiniztion		
Ks8.12	Keratin 13 and 16	Sigma, St. Louis, USA
RKSE60	Keratin 10	Eurodiagnostics, Apeldoorn, NL
6B10	Keratin 4	Eurodiagnostics, Apeldoorn, NL
1C7	Keratin 13	Eurodiagnostics, Apeldoorn, NL
Inflammation		
Anti-elastase	Elastase (polymorphonuclear leucocytes)	Serotec, Oxford, UK
T11	CD2 (T-lymphocytes)	Dakopatts, Copenhagen, DU
WT14	CD14 (monocytes, macrophages)	Dept. Nephrology, Nijmegen, NL
OKT6	CD1a (Langerhans cells)	Ortho Diagnostics Systems, Raritan, USA

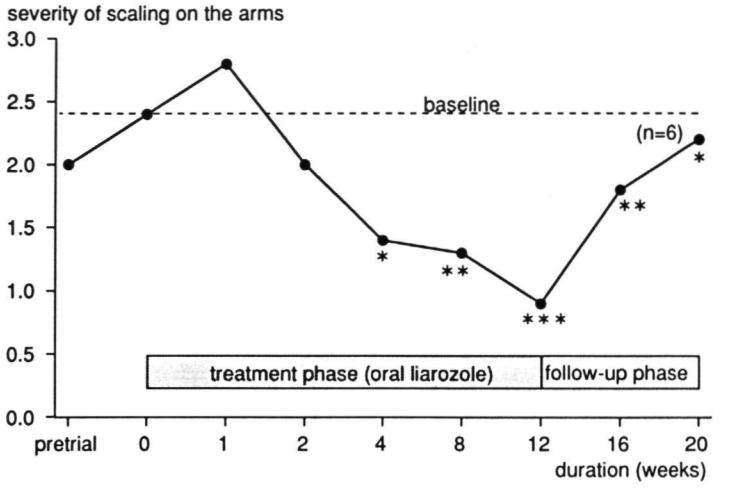


Fig 1. Clinical severity; severity of scaling on the arms worsens during the wash-out period and the first week of treatment. Afterwards a gradual improvement, reaching its maximum at week 12 is observed. Discontinuation of the treatment is followed by progressive worsening of this symptom to pretreatment values at week 8. Whereas all 12 patients were evaluated until week 16, only 6 of them were considered at week 20, because the others required treatment. For the severity of scaling on trunk and legs, as well as the severity of roughness and hyperpigmentation on arms, trunk and legs, a similar pattern is observed. *$p<0.05$ **$p<0.005$ ***$p<0.001$

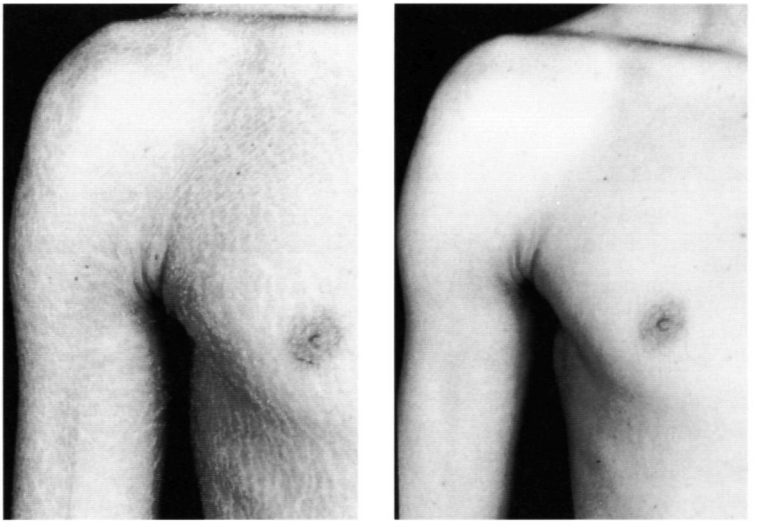


Fig 2 a. NELI-patient before treatment, displaying large brown-coloured scales, covering a substantial part of the body. **b.** After 12 weeks of treatment, a marked reduction of scaling is observed, resulting in a normal appearance.

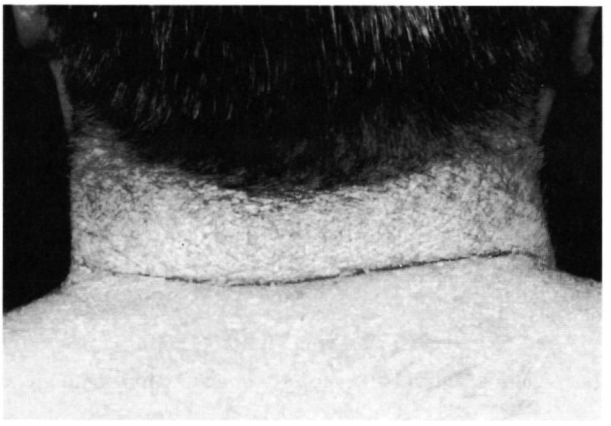
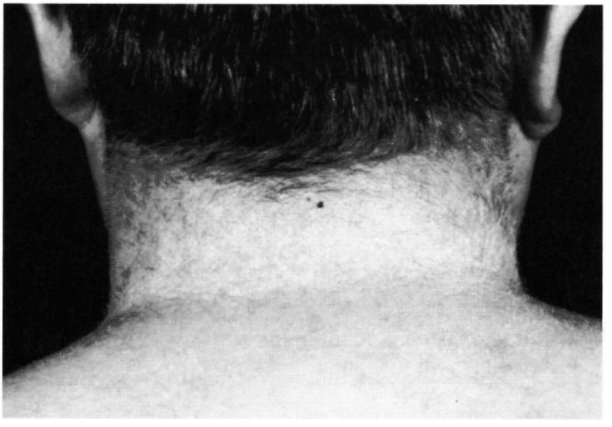


Fig. 3 a. BCIE-patient before treatment, showing thick yellowish scales in the neck, on an erythematous skin.



b. At the end of the treatment phase, a substantial reduction of scales is observed. Some erythema is still present.

Table II. Modulation of expression of keratin 4, 13, and 16, in the various ichthyosis subgroups, under influence of liarozole therapy.

parameter	XRI (n=5)	NELI (n=4)	ELI (n=1)	Brocq (n=2)
keratin 4 (6B10)				
unchanged	1	1	0	0
increased	4	3	1	2
keratin 13 (1C7)				
unchanged	5	4	0	1
increased	0	0	1	1
keratin 13/16 (Ks8.12)				
reduced	0	0	1	0
unchanged	1	3	0	1
increased	4	1	0	1

The most obvious histochemical effect was seen for the expression of keratin 4 (Table II). While keratin 4 was absent at baseline, it was expressed in 10 out of the 12 patients after 12 weeks of treatment, independently of the type of ichthyosis. The induction of keratin 4 was statistically significant ($p<0.05$). Keratin 13 could not be detected at baseline, but was sporadically detected in 2 patients after 12 weeks of treatment. The expression of keratin 13 and 16 was variable: increased in 6 patients, reduced in one patient, and inconsistent in 5 patients. Treatment with liarozole did not change the baseline expression of keratin 10 which could be detected within the suprabasal compartment. Before treatment, the average number of actively cycling cells (mab Ki-67) was 88.9 per mm of section. After 12 weeks of treatment, this number was 86.6 ($p<0.10$). None of the inflammatory parameters did significantly change upon treatment.

DISCUSSION

Twelve patients with different types of ichthyosis, showed a statistically significant improvement in the extent and severity of the skin lesions after 12 weeks of treatment with liarozole. As anticipated clinical side effects were consistent with those experienced in hypervitaminosis A namely: mostly dry lips, dry eyes and itching. These effects were mild and did not necessitate discontinuation of the treatment. No significant changes were observed in the haematological, biochemical and urinary parameters. The present results suggest that liarozole provides a substantial clinical efficacy with limited side effects.

The treatment of disorders of keratinization has been revolutionized by systemic retinoids [14]. However, the margin between safety and efficacy is limited for these drugs. Based on 12 patients, however, it is virtually impossible to conclude whether the therapeutic window between efficacy and side effects is broader for liarozole compared to acitretin. However, the present study suggests that liarozole is at least as effective, whereas the side effect profile is rather similar to that of acitretin. In particular the mucocutaneous side effects, occurring within the therapeutic dose-range is a characteristic for both treatments. Furthermore, dose dependent blistering occurs both during liarozole and acitretin treatment in BCIE [15]. In our opinion double blind comparative study between liarozole and acitretin is warranted in patients with ichthyosis. The development of a topical liarozole formulation could help to broaden the margin between efficacy and side effects even more.

Although the clinical response in various disorders of keratinization and the mucocutaneous side effects suggest that liarozole might work via a retinoid effect, further evidence for such an effect might be provided by immunohistochemical studies on the cellbiological effects during treatment. Immunohistochemical assessment of biopsies, revealed a statistically significant induction of the expression of keratin 4 in 10/12 patients ($p<0.05$). In 2 patients keratin 13 was induced. Keratin 4 and 13 are keratins which are absent in normal adult human skin. Induction has been described after

treatment with topical retinoids [16,17]. Therefore, the present results provide indirect evidence for the hypothesis that the pharmacological effects of liarozole could be mediated by enhanced levels of endogenous retinoic acid. However, further studies on retinoid levels in the skin and induction of expression of cellular retinoic acid binding protein II are required before this hypothesis can be proven.

Theoretically, topical application of liarozole may be effective too. However, at the start of this study topical liarozole was not available. Furthermore, topical retinoids used in the past were characterized by a high irritancy potential [18]. Based on the good clinical results obtained with oral liarozole in this study, a topical liarozole formulation has been developed. Studies on topical liarozole in ichthyosis are currently ongoing.

In conclusion, in this open study, treatment with liarozole was followed by a substantial clinical improvement in all patients with ichthyosis. Relevant laboratory side effects were absent. Subjective side effects were reminiscent of those observed with synthetic retinoids. The immunohistochemical results lend further support for a retinoid mechanism, although other modes of actions are not excluded. Additional controlled studies are required to confirm the efficacy and to determine the optimum dosage and safety profile of this new compound.

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REFERENCES

1. Dowling JE, Wald G. The biological function of vitamin A acid. Proc Nat Acad Sci USA 1960;46:587-608.
2. Chytil F. Retinoic acid: Biochemistry, pharmacology, toxicology and therapeutic use. Pharmacol Rev 1984;36:93-100.
3. Wolf G. Multiple functions of vitamin A. Physiol Rev 1984;64:873-937.
4. Pilkington T, Brogden RN. Acitretin. A review of its pharmacology and therapeutic use. Drugs 1992;43:597-627.
5. Blanchet-Bardon CI, Nazarro V, Rognus C, Geiger JM, Puissant A. Acitretin is the treatment of severe disorders of keratinization. Results of an open study. A Am Acad Dermatol 1991;24:982-986.
6. Di Giovanni JJ, Helfgott RK, Gerben LH. Extraspinal tendon and ligament calcification associated with long-term therapy with etretinate. N Eng J Med 1986;315:1177-82.
7. Baden HP, Buxuan MM, Weinstein GD. Treatment of ichthyosis with isotretinoin. J Am Acad Dermatol 1982;6:716-20.
8. Ellis CN, Voorhees JJ. Etretinate therapy. J Am Acad Dermatol 1987;16:267-91.
9. Wauwe J van, Coene MC, Goossens J, et al. Effects of cytochrome P450 inhibitors on the in vivo metabolism of all trans-retinoic acid in rats. J Pharmacol Exp Ther 1990;252:365-9.
10. Bruynseels J, Coster R de, Rooy P van, et al. R 75251, a new inhibitor of steroid biosynthesis. The Prostate 1990;16:345-57.

11. Coster T de, Coene MC, Wauwe J van, et al. Assessment of the multiple-dose pharmacokinetics of liarozole fumarate and its effect on the endocrine system in healthy male subjects. Clinical Research Report. Janssen Research Foundation. N 106593/1 May 1994.
12. Doncker P, Deree J, Degreef H. Inhibition of the metabolism of endogenous retinoic acid as treatment for severe psoriasis: an open study with oral Liarozole. Br J Dermatol 1995;133:426-432.
13. Lucker GPH, van de Kerkhof PCM, Castelijns FACM, van Dijk MR, Schalkwijk LJM, Steijlen PM. Topical treatment of ichthyoses with 13-cis retinoic acid. A clinical and immunohistochemical study. Eur J Dermatol 1995;5:566-71.
14. Fritsch PO. Retinoids in psoriasis and disorders of keratinization. J Am Acad Dermatol 1992;27:S8-S14.
15. Steijlen PM, Dooren-Greebe RJ van, Happle R, Kerkhof PCM van de. Ichthyosis bullosa of Siemens responds well to low-dosage oral retinoids. B J Dermatol 1991;125:469-71.
16. Steijlen PM, Happle R, Muijen GNP van, et al. Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. Dermatologica 1991;182:178-83.
17. Rosenthal DS, Griffiths EM, Yuspa SH, et al. Acute or chronic topical retinoic acid treatment of human skin in vivo alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10, and 14. J Invest Dermatol 1992;98:343-50.
18. Orfanos CE, Ehlert R, Gollnick H. The retinoids, a review of their clinical pharmacology. Drugs 1987;34:459-503.

Chapter 6

Topical liarozole treatment of hereditary ichthyoses: a double-blind, placebo-controlled left-right comparative clinical and immunohistochemical study.

GPH Lucker
AMC Heremans
PJ Boegheim
PM Steijlen

Submitted.

SUMMARY

The novel imidazole derivative liarozole, inhibits the cytochrome P450-dependent 4-hydroxylation of retinoic acid, increasing the tissue levels of endogeneous retinoic acid. After a wash-out phase of 2-4 weeks, twelve male patients with ichthyosis were treated for 6 weeks, using a bilaterally paired double-blind approach with liarozole 5% cream. This was followed by an open phase of 4 weeks during which both sides were treated with liarozole cream. Immunohistochemical parameters of epidermal keratinization and inflammation were assessed before and after the double-blind treatment. At the end of the double-blind treatment, a significant unilateral improvement in extent and severity of skin lesions was observed in favour of the liarozole cream treated side. Clinical side effects were only minimal. No relevant changes were found in the haematological, biochemical, and urinary parameters. Plasma levels of liarozole could be detected in 10 patients. Highest levels were observed in those with more inflammatory types of ichthyosis. However, except for one patient, these levels were apparently not therapeutical, as only the side treated with liarozole cream responded well to the treatment. On the other hand, decreased oestradiol levels were measured in some patients, which might have been due to a systemic effect of liarozole, but without correlation between oestradiol and liarozole levels. Finally, immunohistochemistry showed induction of keratin 4 in 3 patients and of keratin 13 in one patient at the end of the double blind treatment, again only on the side treated with liarozole cream. At the end of the open treatment, an inverted clinical effect was observed. Unilateral improvement in extent and severity was noticed in favour of the previously cream base treated side. Topical administration of liarozole 5% cream caused a definite amelioration in different types of ichthyosis, suggesting that therapeutical concentrations of retinoic acid may be achieved by an in situ inhibition of the endogeneous breakdown of this acid. The retinoid-like effect on the keratin pattern was only seen on the liarozole-treated side, further indicating a selective increase of retinoic acid in the target skin cells.

INTRODUCTION

The genetically determined ichthyoses require lifelong treatment. Whereas topical treatment is the therapy of first choice, most topical therapeutical modalities available, however, are insufficient as monotherapy in the treatment of severe ichthyosis subtypes.

Retinoids (vitamin A-acid derivatives) are important compounds that control proliferation and differentiation of epithelial tissues in mammals (1-4). Liarozole, a novel imidazole derivative, inhibits the cytochrome P450-dependent 4-hydroxylation of retinoic acid which is the physiological breakdown of all-trans-retinoic acid (5,6). In vitro pharmacological studies in cultured human breast cancer cells, teratocarcinoma cells, and keratinocytes, demonstrated a synergistic effect of liarozole and exogenously added retinoic acid (RA) (7,8). In-vivo keratinization and tumour models in rats and mice showed that orally administered liarozole (10-40 mg/kg/day) delays the plasma clearance

and enhances tissue levels of endogenous RA, and mimics the biochemical and morphological effects of RA on proliferation and differentiation of epithelial tissues (9,10). The presently available clinical data corroborate these in-vitro and in-vivo data on RA metabolism, as oral administration of liarozole proved efficacious in the treatment of psoriasis (11), and cancer patients receiving oral liarozole developed cutaneous signs of hypervitaminosis A (12).

Besides inhibiting the 4-hydroxylation of RA, liarozole also inhibits cytochrome P450-dependent steps in the biosynthetic pathways of testicular, ovarian and adrenal steroids, mainly the conversion of androgens to estrogens (aromatase), progestins to androgens (17-hydroxylase, 17-20-lyase) and of 11-deoxycorticosterone to corticosterone (11-hydroxylase) (6). However, the effect on testicular and adrenal steroid biosynthesis may not be clinically relevant, as upon chronic administration of oral liarozole in human volunteers, recovery of plasma testosterone and cortisol levels was achieved by hypopituitary compensation (13). In accordance, psoriasis patients receiving oral liarozole treatment did not reveal abnormalities in cortisol and testosterone levels (14).

Topical therapeutical modalities available in ichthyosis mainly consist of urea and lactic acid, aiming at rehydration and keratolysis of the cornified cell layer. However, these ointments are of limited value as they are only effective in autosomal dominant ichthyosis vulgaris, whereas the more severe ichthyosis subtypes do not improve sufficiently. Recently, we demonstrated a good clinical improvement of lamellar and epidermolytic ichthyosis with topical calcipotriol (15). However, to prevent derangements in calcium metabolism, the maximum weekly amount has been limited to 100 grammes. This limitation constitutes a restriction for its use, as in these severe ichthyosis patients, a substantial part of the total body surface is affected. The use of topical retinoids has been limited because of irritative side effects (16). However, 13-cis-retinoic acid constitutes an exception to this rule, and might be useful in the topical treatment of ichthyosis (17).

The present study was performed to investigate the effectiveness and tolerability of liarozole 5% cream in 12 patients with hereditary ichthyoses. Immunohistochemical parameters of epidermal differentiation and inflammation were assessed before and after treatment.

PATIENTS AND METHODS

In a randomized, double blind, placebo controlled, left-right comparative study, 12 patients with a hereditary ichthyosis, were treated with topical liarozole (liarozole fumarate) 5% cream and the cream base only. The following ichthyoses could be distinguished: 6 patients with X-linked recessive ichthyosis (XRI), 4 with non erythrodermic lamellar ichthyosis (NELI), and 2 patients with congenital bullous ichthyotic erythroderma of Brocq (CBIE). After a wash-out phase (2-4 weeks) the lesions were treated at one side with liarozole 5% cream and at the contralateral side with the cream base only for a period of 6 weeks. In an open follow-up phase, both sides of the

body were treated with liarozole cream for 4 weeks. The creams were applied thinly and evenly, twice daily. Written informed consent was obtained after the nature of the procedures had been fully explained. XRI-patients had a documented steroidsulphatase deficiency, and sun exposure had to be avoided. Patients were examined before treatment, at week 0,2,4,6 of the double-blind treatment phase, and at the end of the open treatment. At each visit, severity and extent of the skin lesions was assessed by the investigator. The severity of involvement was rated using a 4 point scale for scaling, roughness, hyperpigmentation, erythema, and bullae, as follows: 0 = absent, 1 = mild , 2 = moderate, 3 = severe. Scaling and roughness were recorded in all subgroups, hyperpigmentation only in XRI, erythema and bullae in CBIE. The extent of involvement was recorded for the left and right side of the upper and lower extremities separately and expressed in % of the surfaces of these body parts, going from 0 to 100%. An overall response to treatment was documented by the investigator and the patient at each visit for severity and extent of involvement, on a five-point scale: worse, unchanged, slightly improved, markedly improved, and cleared.
Haematological, biochemical, urinary, and endocrinological examinations assessing cortisol, testosterone, oestradiol, and luteinizing hormone, were performed at week 0, 6 and 10. Plasma samples for drug level analysis were taken at week 6 and 10 of the treatment. At the start and the end of the double-blind treatment phase, a photographic documentation was done under standardized conditions. Statistical analysis was performed using the Wilcoxon signed-rank test for paired data.

Biopsies from representative skin lesions were taken, one at the start and one from each side at the end of the double-blind treatment phase. The biopsies were snap frozen in liquid nitrogen and stored at -80°C until processing. Slices of 4 µm were cut and fixed in acetone for 10 minutes. Staining to assess epidermal keratinization, and epidermal and dermal inflammation, was done, using the following antibodies:
Keratinization
Ks8.12 (Sigma, St Louis, USA): directed against keratin 13 and 16. Keratin 13 is a fetal cytokeratin, not present in adult human skin, induced after retinoid treatment. Keratin 16 is expressed by hyperproliferative keratinocytes.
RKSE60 (gift of F. Ramaekers, Dept. of Medicine, Pathology, University Hospital Maastricht): binding keratin 10, expressed in differentiated epidermis.
6B10 (Eurodiagnostics, Apeldoorn, Netherlands): directed against keratin 4. Keratin 4 is another cytokeratin, not present in adult human skin, induced after retinoid treatment.
1C7 (Eurodiagnostics, Apeldoorn, Netherlands): binding keratin 13.
Inflammation
Anti-elastase (Serotec, Oxford, England): staining leucocyte elastase, present in polymorphonuclear leucocytes.
T11 (Dakpopatts, Copenhagen, Denmark): staining CD2, present on T-lymphocytes.

WT14 (Dept. of Medicine, Nephrology, University Hospital Nijmegen): directed against CD14, present on monocytes and macrophages.
OKT6 (Ortho Diagnostic Systems, Raritan, USA): staining CD1a, present on Langerhans cells.
An indirect alkalic phosphatase technique was used for staining with the antibodies Ki-67 and anti-elastase. Slides were incubated for 60 minutes with the primary antibodies. After washing in phosphate buffer saline (PBS) the slides were incubated with a solution of rabbit-anti-mouse-immunoglobulin conjugated with alkalic phosphatase for 30 minutes. After washing in PBS, slides were stained with fast red, counterstained with Mayer's haematein and mounted in glycerin gelatin.
Staining with Ks8.12, RKSE60, 6B10, 1C7, T11, OKT6 and WT14, was carried out using an indirect peroxidase technique. Slides were incubated for 60 minutes with the primary antibody. After washing in PBS, the slides were incubated with a solution of rabbit-anti-mouse-immunoglobulin conjugated with peroxidase for 30 minutes. After washing in PBS, the peroxidase label was visualized by incubation with 3.3 diaminobenzidintetrahydrochloride (DAB) solution as a substrate. Subsequently, they were incubated with CuSO4 for 5 minutes, counterstained with Mayer's haematein, dehydrated and mounted in permount. The whole procedure was performed at room temperature.
Presence in the epidermis and dermis of the stained structures was assessed using a semi-quantitative scale:
Epidermis: 0= no staining; 1= sporadic staining; 2= minimal staining; 3= moderate staining; 4= moderate-pronounced staining; 5= pronounced staining; 6= whole epidermis stained.
Dermis: 0= no presence; 1= sporadically present; 2= 1-25% of infiltrate cells stained; 3= 26-50% of infiltrate cells stained; 4= 51-75% of infiltrate cells stained; 5= 76-99% of infiltrate cells stained; 6= 100% of infiltrate stained.

RESULTS

Extent and severity of skin involvement, assessed by the investigator, are summarized in Table I, and Figure 1, 2, and 3.
Wash-out phase. During the wash-out phase, the extent and severity of the lesions hardly changed.
Double-blind treatment phase. At the start of the double-blind treatment phase, the extent of skin lesions on the upper extremities was 87%. At the end of this phase, the extent was reduced to 44 % at the liarozole treated side ($p=0.001$) and 69% at the placebo treated side ($p<0.005$). The difference in reduction between both sides was statistically significant in favour of the liarozole treated side ($p<0.005$). The severity of scaling and roughness was strongly reduced at the liarozole treated side (scaling $p=0.0005$; roughness $p=0.0005$), but not at the placebo treated side. Also in this respect,

analysis of the difference between both sides revealed that liarozole was superior to placebo (scaling p=0.0005; roughness p=0.0005).

Follow-up phase. After 4 weeks of bilateral treatment with liarozole, a considerable reduction of the extent of skin lesions (-24%) was found at the previously placebo treated arms ($p<0.01$), whereas only a moderate reduction (-11%) was found at the contralateral side ($p<0.1$). Analysis of the difference in shifts between both sides, displayed an improvement in favour of the previously placebo treated side ($p<0.05$). With regard to severity, the previously placebo treated arms showed a reduction of scaling ($p<0.005$) and roughness ($p<0.005$), which was absent at the contralateral side. Comparing both sides, again revealed improvement in favour of the previously placebo treated side (scaling $p<0.005$; roughness $p<0.005$).

As shown in Table I, the development of extent and severity of lesions on the legs in both double-blind treatment and follow-up phase, parallel the shifts described for the arms.

Table I. Extent and severity of skin lesions. Statistical testing on the difference in the change between treatment sides, compared to baseline values: * $p\leq0.05$ ** $p\leq0.01$ *** $p\leq0.001$. The asterisks are placed in the column corresponding to the side of the body where the improvement was statistically significantly better.

	liarozole side	placebo side
extent of lesions on arms (%)		
week 0	86%	86%
week 6	44%**	69%
week 10	33%	45%**
extent of lesions on legs (%)		
week 0	87%	87%
week 6	57%**	74%
week 10	48%	58%*
severity of scaling on arms		
week 0	2 50	2.50
week 6	1 08***	2.33
week 10	1.00	1.36**
severity of roughness on arms		
week 0	2.50	2.50
week 6	1.08***	2.33
week 10	1.00	1 36**
severity of scaling on legs		
week 0	2 67	2.67
week 6	1.50**	2.33
week 10	1.45	1.82
severity of roughness on legs		
week 0	2 67	2 67
week 6	1.50**	2.33
week 10	1.45	1.82

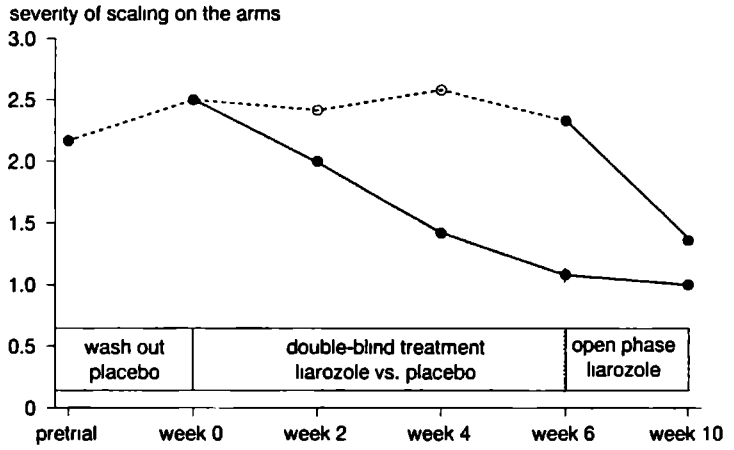


Fig. 1 The severity of skin lesions shows definite improvement in favour of the liarozole treated side during the double-blind treatment phase, whereas a reversed effect is observed during the open treatment phase.

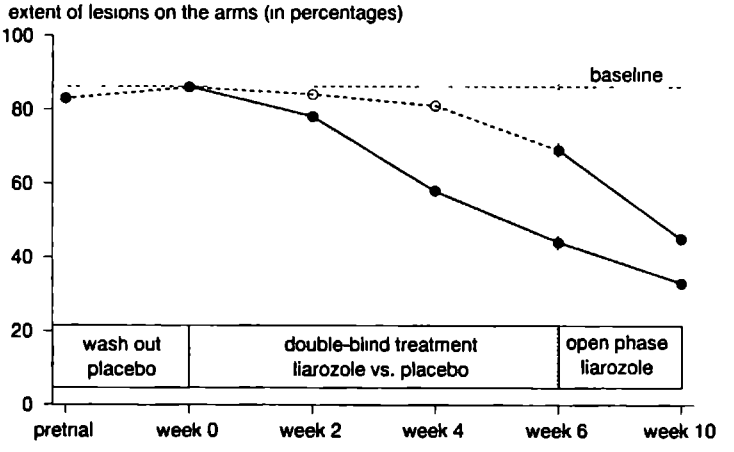
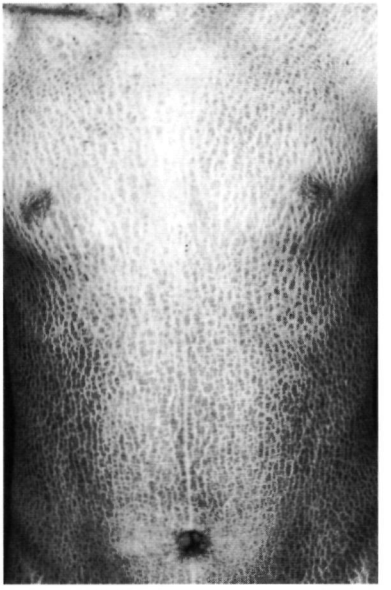
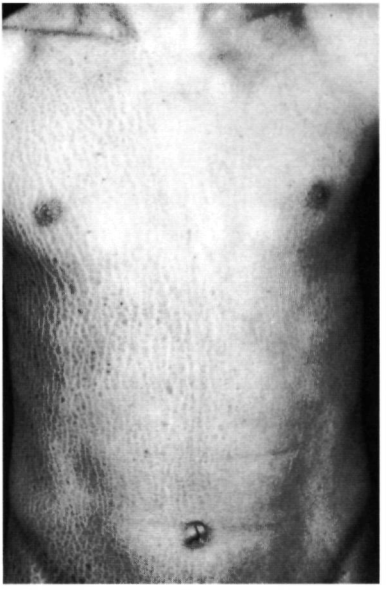


Fig. 2 In the double-blind treatment phase, reduction of skin lesions on the arms is considerable (86 to 44%) at the liarozole treated side, whereas improvement is only modest at the cream base treated side (86 to 69%).



a



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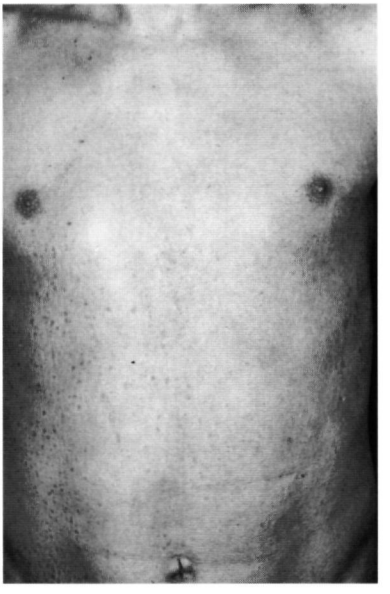

c

Fig. 3
a. NELI patient before treatment, exhibiting large brown scales covering a substantial part of the skin surface.
b. NELI patient at the end of the double-blind treatment, showing marked improvement at the liarozole treated left side, whereas improvement at the cream-base treated right side is only small.
c. After 6 weeks double-blind and 4 weeks open liarozole treatment, improvement is marked on both sides. At the previously cream-base treated right side, minimal scaling is still visible.

Adverse experiences, were mentioned by 6 patients, and are summarized in Table II. In 5 patients, they are probably liarozole related, since they were present only at the liarozole treated side. Two patients stopped treatment because of adverse experiences. One patient stopped at the end of the double-blind treatment phase because of erythematous itching papules at the liarozole treated side, probably due to liarozole. Another patient stopped

treatment during the follow-up phase after 8 weeks of treatment because of contact eczema, which started already during the wash-out phase, and therefore probably related to the cream-base. No significant changes of haematological, biochemical, and urinary parameters were found. Endocrinological examinations revealed after 0, 6, and 10 weeks of treatment, a reduction in the plasma oestradiol concentration. No relevant changes were found in all other hormones tested (cortisol, testosterone, LH). Drug plasma concentrations of liarozole could be measured in 10 patients after 6 and 10 weeks of treatment and varied strongly (Figure 4). No correlation could be established between the liarozole and oestradiol plasma concentrations. The plasma concentration seems to be related to the type of ichthyosis. The highest concentrations were found in patients with NELI, the lowest in patients with XRI. Only the patient with the highest plasma concentration showed a marked improvement at the placebo treated side during the double-blind phase. This was the only patient who had completely cleared arms at the end of the follow-up phase.

Table II. Per trial phase, the side of the body (liarozole treated or placebo treated) of the adverse experience is mentioned. (A) dose reduction, (B) temporary stop, treatment continuation, permanent stop, (C) temporary stop, (D) dose reduction, (E) permanent stop.

Table II: Survey of adverse experiences.

pat. no.	Adverse experience	wash-out phase	double-blind phase	open phase
1	ITCH ON TRUNK		liarozole (A)	liarozole
2	DRY LIPS			liarozole
3	- ITCH - ERYTHEMATOUS PAPULES ON ARM - ITCHING ERYTHEMATOUS PAPULES, LEG		liarozole (B)	
4	BILATERAL CONTACT ECZEMA ON NATES, UPPER LEGS AND ABDOMEN	placebo (C)	placebo + liarozole (D)	liarozole (E)
5	ITCH ON TRUNK		liarozole	
9	PARAESTHESIA AFTER APPLICATION OF CREAM		liarozole	

Per trial phase, the side of the body (liarozole-treated or placebo-treated) of the AE is mentioned.
(A) dose reduction
(B) temporary stop, treatment continuation, permanent stop
(C) temporary stop
(D) dose reduction
(E) permanent stop

Staining for keratin 4 and 13 was absent at baseline. After 6 weeks of treatment, a slight induction of keratin 4 was found in 3 patients (1 NELI, 2 CBIE) patients, and an induction of keratin 13 in one CBIE patient. Expression was only found at the liarozole treated side. Treatment with liarozole did not change the baseline expression of keratin 10 which could be detected within the suprabasal compartment. None of the inflammatory parameters did significantly change upon treatment.

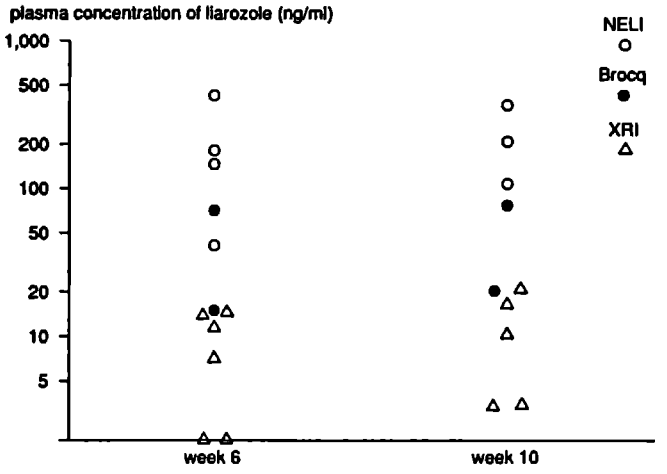

Fig. 4 A remarkable difference is observed in liarozole plasma concentrations, measured after 6 and 10 weeks of treatment. Concentrations appear rather high in NELI, moderate in CBIE, and low in XRI.

DISCUSSION

In this double-blind, placebo-controlled trial, it has been demonstrated that liarozole 5% cream is effective in various forms of ichthyosis (XRI, NELI, CBIE). After 4 and 6 weeks of treatment, the extent of the lesions and the severity of scaling was statistically significantly more reduced at the liarozole-treated side than at the placebo-treated side. This liarozole-induced improvement was robust, so statistically significant differences with placebo were already obtained in a small trial with 12 patients. The effectiveness of liarozole was also evident in the follow-up phase, were both sides of the body were treated with liarozole. In the follow-up phase, the largest improvement was found at the previously placebo-treated side, reflecting a catching-up effect. Overall, the spectrum of side effects of topical liarozole is similar or milder to that of calcipotriol or topical retinoids. Nevertheless, two patients discontinued treatment because of side effects. In one case, side effects were related to the cream base rather than to liarozole. Liarozole is known to be an inhibitor of estrogen biosynthesis. In this trial a reduction of the plasma estradiol concentration was found after topical administration of liarozole. However, the amount of estradiol reduction was not related to the plasma concentration of liarozole.

Liarozole is clinically effective by its inhibition of the cytochrome P450- dependent break-down of endogenous retinoic acid. Consequently, liarozole increases tissue levels of retinoic acid. Topical liarozole induces the fetal keratins 4 and 13 at the liarozole treated side only. This induction is probably a consequence of the increase in the amount of endogenous retinoic acid, for induction of keratins has also been found after topical administration of retinoids (18,19).

In this trial we discovered that administration of liarozole cream resulted in rather high plasma concentrations in patients with NELI or CBIE, but less in patient with XRI. Between these groups no significant differences were found in the amount of liarozole used, so the differences in plasma concentrations might be explained by skin differences, resulting in differences in the absorption of liarozole. NELI and CBIE represent hyperproliferative ichthyosis subtypes (20). Consequently, the demand for nutrients and therefore dermal blood supply, are probably higher compared to subtypes characterized by normal proliferation, such as XRI. This might explain the differences in liarozole absorption. The highest plasma concentrations measured after topical administration could be clinically relevant: the strongest improvement at the placebo-treated side was found in the patient with the highest plasma level of liarozole. This NELI patient had levels comparative to the peak plasma levels found in healthy volunteers after a single oral dose administration of 37.5 mg.

In conclusion, liarozole 5% cream is effective in the treatment of various ichthyosis subtypes. The clinical improvement is probably achieved by a local inhibition of the endogenous breakdown of retinoic acid. Consequently the concentration of retinoic acid is elevated specifically in the target epidermal cells, minimalizing the risk of systemic effects, as illustrated by the good tolerance in this trial. Liarozole plasma concentrations could be measured indicating systemic resorption of liarozole. However, as consistent clinical improvement and biochemical induction of keratins were only observed at the liarozole treated side, circulating retinoic acid must be low or absent. Plasma liarozole levels might exert hormonal effects, as the oestradiol levels were decreased in some patients. These however were not correlated with the liarozole plasma concentrations, and patients did not experience hormonal side effects. These were also lacking during oral liarozole treatment of ichthyosis (21) and psoriasis (11).

Therefore, liarozole 5% cream represents a new concept which is of interest as it might represent a clinical effective and safe therapeutical option without systemic retinoic acid effects. As a certain degree of systemic resorption of liarozole is present, future studies remain to be performed to establish safety parameters.

ACKNOWLEDGEMENTS

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REFERENCES

1. Dowling JE, Wald G. The biological function of vitamin A acid. Proc Nat Acad Sci USA 1960;46:587-608.
2. Chytil F. Retinoic acid: Biochemistry, pharmacology, toxicology and therapeutic use. Pharmacol Rev 1984;36:93-100.
3. Wolf G. Multiple functions of vitamin A. Physiol Rev 1984;64:873-937.
4. Pilkington T, Brogden RN. Acitretin. A review of its pharmacology and therapeutic use. Drugs 1992;43:597-627.
5. van Wauwe J, Coene MC, Goossens J, van Nijen G, Cools W, Lauwers W. Effects of cytochrome P-450 inhibition on the in vivo metabolism of all-trans-retinoic acid in rats. J Pharmac Exp Ther 1990;252:365-9.
6. Bruynseels J, Coster R de, Rooy P van, et al. R 75251, a new inhibitor of steroid biosynthesis. Prostate 1990;16:345-357.
7. Wouters W, van Dun J, Dillen A, Coene M, Cools W, de Coster R. Effects of liarozole, a new antitumoral compound, on retinoic acid induced inhibition of cell growth and on retinoic acid metabolism in MCF-7 human breast cancer cells. Cancer-Res 1992;52:2841-6.
8. Williams JB, Napoli JL. Metabolism of retinoic acid and retinol during differentiation of F9 embryonal carcinoma cells. Proc Natl Acad Sci USA 1985;82:4658-62.
9. De Coster R, van Ginckel R, van Moorselaar RJA, et al. Antitumoral effect of R 75251 in prostatic carcinoma: experimental and clinical studies. Proc. Am. Assoc. Cancer Res. 1991;32:213.
10 van Ginckel R, de Coster R, Wouters W, et al. Antitumoral effects of R75251 on the growth of transplantable R3327 prostatic adenocarcinoma in rats. Prostate 1990;16:313-23.
11. Dockx P, Decree J, Degreef H. Inhibition of the metabolism of endogenous retinoic acid as treatment for severe psoriasis: an open study with oral liarozole. Br J Dermatol, accepted.
12. De Coster R, Wouters W, van Ginckel R, et al. Experimental studies with liarozole (R75251): an antitumoral agent which inhibits retinoic acid breakdown. J Steroid Biochem Molec Biol 1992;43:197-201.
13. De Coster R. Assessment of the multiple-dose pharmacokinetics of liarozole fumarate and its effects on the endocrine system in healthy male subjects. Part III:pharmacodynamics. Clinical Research Report, LIA-BEL-22, 1994.
14. Doncker P de, Legendre B, Dhondt A, Cauwenbergh G. Inhibitors of the metabolism of retinoic acid in the skin. A novel approach in the treatment of acne and psoriasis. 50th Annual meeting of the American Academy od Dermatology, Dallas, USA, December 7-12, 1991.
15. Lucker GPH, Kerkhof PCM van de, Dijk MR van, Steijlen PM. Effect of topical calcipotriol on congenital ichthyoses. Br J Dermatol 1994;131:546-550.
16. Orfanos CE, Ehlert R, Gollnick H. The retinoids, a review of their clinical pharmacology. Drugs 1987;34:459-503.
17. Lucker GPH, Kerkhof PCM van de, Castelijns FACM, Dijk MR van, Steijlen PM. Topical treatment of ichthyoses with 13-cis-retinoic acid. A clinical and immunohistochemical study. Eur J Dermatol 1995;5:566-571.
18. Steijlen PM, Happle R, Muijen GNP van, et al. Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. Dermatologica 1991;182:178-183.

19. Rosenthal DS, Griffiths EM, Yuspa SH, et al. Acute or chronic topical retinoic acid treatment of human skin in vivo alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin, and keratins 6 and 13 but not keratins 1, 10, and 14. J Invest Dermatol 1992;98:343-350.
20. Lucker GPH, Steijlen PM, Suykerbuyk EJA, Kragballe K, Kerkhof van de PCM. Flowcytometrical investigation of epidermal cell characteristics in monogenic disorders of keratinization and their modulation by topical calcipotriol treatment. Acta Derm Venereol (Stockh) 1996;76:97-101.
21. Lucker GPH, Heremans AMC, Boegheim PJ, Steijlen PM. Oral treatment of ichthyosis by modulating endogeneous retinoic acid: a new concept for the treatment of keratinization disorders. Br J Dermatol, accepted.

Chapter 7

Topical treatment of Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study.

GPH Lucker
PCM van de Kerkhof
FACM Castelijns
PM Steijlen

J Dermatol Treat. Accepted.

SUMMARY

In a prospective, double-blind, left-right comparative study, a cream containing 13-cis-retinoic acid 0.1% (13-cis-RA) and its cream base were applied on a test patch of skin during a period varying between 3 and 10 weeks in 7 patients with Darier's disease. A half-side reduction of papules was observed in 4 patients. The treatment had no effect on scaling and increased erythema in 2 patients. Clinical improvement of papules was statistically significant, whereas scaling and erythema did not change significantly. Side effects due to the study medication were only minimal and easily controlled by reduction of the application frequency. Biopsies for immunohistochemical examination were taken from representative skin lesions, one before and one from each side after treatment. Keratins 4 and 13, were induced by the treatment with topical 13-cis-RA in 3 and 2 patients respectively.

In conclusion, topical 13-cis-RA 0.1% cream is a well-tolerated therapy for Darier's disease. Induction of keratins 4 and 13 seems to offer an immunohistochemical tool for the investigation of the mode of action of 13-cis-RA.

INTRODUCTION

Darier's disease is an autosomal dominant monogenic disorder of keratinization, characterized by changes in epidermal differentiation and increased proliferation. Oral application of retinoids is very effective in alleviating Darier's disease (1). Because of the risk of systemic side effects, topical application would be preferable. Beneficial effects of topical treatment with all-trans-retinoic acid in disorders of keratinization, have been documented (2-6). Irritation of the skin, however, has limited its use to the treatment of acne. Compared to the intolerable tenderness caused by treatment with all-trans-retinoic acid, irritation induced by treatment with 13-cis-RA is negligible. Recently, good clinical results were observed in Darier's disease with 13-cis-RA 0.1% (7,8,9). The aim of the present study was to evaluate the clinical efficacy of prolonged topical application of 13-cis-RA 0.1% in 7 patients with Darier's disease. Furthermore, immunohistochemical assessment was made of parameters of proliferation, keratinization, and inflammation, using monoclonal antibodies.

PATIENTS AND METHODS

Patients

Seven patients with Darier's disease were treated with topical 13-cis-retinoic acid after informed consent was obtained. Details regarding sex, age, duration of treatment, reason for discontinuation, and side effects are summarized in Table I. Topical as well as systemic treatment was stopped respectively 2 and 4 weeks before the study medication was started. In every patient two comparative and representative test areas of 10x10 cm were selected. In this prospective, double-blind, left-right comparative study, a cream

containing 13-cis-RA in 0.1% concentration and the cream base only (Hofmann-la Roche, Basel, Switzerland) were applied during a period varying between 3 and 10 weeks. Patients were instructed to apply the creams thinly and evenly twice daily. In case of intolerable irritation, they were advised to reduce the application frequency. Papules, scaling, and erythema were scored using a 4 point scale, in order to establish clinical efficacy. Statistical analysis was carried out using the Wilcoxon signed-rank test for paired data.

Table I. Patient details and tolerability to 13-cis-RA

patient	sex	age	duration of treatment (weeks)	reason for discontinuation of trial medication	side effects 13-cis-RA side	side effects cream base side
1	F	43	10	end of study	burning, itching	none
2	F	52	3	worsening of Darier	irritation	irritation
3	M	20	10	end of study	none	none
4	M	19	10	end of study	itching	none
5	M	28	8	worsening of Darier	itching	irritation
6	F	49	6	clearance of skin lesions at the cream-base treated side	none	none
7	M	21	10	end of study	none	none

Analytical procedures

Biopsies from representative skin lesions within the test areas were taken, one at the start and one from each area at the end of treatment. To assess epidermal proliferation, keratinization and epidermal and dermal inflammation, the slides were stained using the monoclonal antibodies summarized in Table II. Staining procedures were carried out as described previously (10).

RESULTS

Clinical response

Patient details and side effects of the treatment with 13-cis-RA and the cream base are summarized in Table I. Table III provides the efficacy results for the individual patients. At the end of the study, a half-side reduction of papules was observed in 4 out of 7 patients in favour of the 13-cis-RA treated side (patient 1, 3, 4, and 7). Patient 5 demonstrated a clear half side effect in favour of the 13-cis-RA treated side after 4 weeks, followed by a relapse of the disease on both sides, requiring systemic treatment with acitretin. Patient 2 did not show a half side effect. She was withdrawn from the study because of a severe relapse requiring systemic treatment with acitretin. Half-side improvement of scaling in favour of the 13-cis-RA treated side, was only observed in

patient 3. Patient 6 displayed a remarkable improvement of both induration and scaling at the cream-base treated side, whereas improvement was only moderate at the 13-cis-RA treated side. In 2 of the 4 responders, worsening of erythema was observed on the 13-cis-RA treated side. The reduction of papules at the 13-cis-RA treated side compared to the cream base side was statistically significant ($p<0.05$). The slight increase of erythema was not statistically significant.

As side effects, burning and itching were reported in 1 and 3 patients respectively. These were not severe and diminished by reducing the treatment frequency.

A considerable interindividual variation concerning the duration of treatment necessary for reduction of skin lesions was noticed. Of the 4 responders, 2 did not improve until the second month of treatment. In one of the 2 patients responding in the first month, further improvement was achieved by continuing the treatment.

Table II. Antibodies used in the study

Monoclonal antibody	Antigen	Location of antigen
6B10	cytokeratin 4	foetal skin and stratifying, non-cornifying
1C7	cytokeratin 13	(suprabasal) cells
2D7	cytokeratin 13	
RCK102	cytokeratin 5 (and 8)	basal cells
RCK107	cytokeratin 14	
LL002	cytokeratin 14	
RCK105	cytokeratin 7	simple epithelial cells
Cam5.2	cytokeratin 8	
M20	cytokeratin 8	
LE4.1	cytokeratin 8	
RGE53	cytokeratin 18	
RCK106	cytokeratin 18	
CK18.2	cytokeratin 18	
2C8	cytokeratin 18	
LP2K	cytokeratin 19	
RCK108	cytokeratin 19	
Ks8.12	cytokeratin 13 and 16	foetal skin and hyperproliferative keratinocytes
RKSE60	cytokeratin 10	stratifying, cornifying suprabasal cells
		cycling cells
Ki67	nuclear antigen	B-lymphocytes
LEU14	CD22	Langerhans cells
OKT6	CD1A	macophages and monocytes
WT14	CD14	T-lymphocytes
T11	CD2	polymorphonuclear leucocytes
anti-elastase	elastase	

Histological findings

The markers for keratin staining patterns did not show consistent changes, except for the monoclonal antibodies 6B10 and 1C7, staining keratin 4 and 13. Whereas staining was absent before treatment, staining for keratin 4 and 13 (mab 1C7) was observed in 3

(patient 1, 3, and 4) and 2 (patient 1 and 2) patients respectively, after treatment (Fig. 1). Staining was only found at the 13-cis-RA treated side. The average number of cycling cells per millimetre epidermis is shown in Table IV. No changes were found for the investigated parameters of inflammation.

Table III. Clinical effects of topical 13-cis-RA

Patient no.	Vehicle			13-cis-RA		
	start	4wks	end	start	4wks	end
Effect on induration						
1	3	2	3	3	2	1
2	2	*	3	2	*	3
3	2	2	2	2	2	1
4	3	3	3	3	1	1
5	2	2	2	2	0	2
6	3	1	0	3	2	2
7	2	2	2	2	2	1
Effect on scaling						
1	1	0	0	1	0	0
2	1	*	1	1	*	1
3	1	1	1	1	1	0
4	0	0	0	0	0	0
5	1	1	2	1	1	1
6	3	0	0	3	2	1
7	0	0	0	0	0	0
Effect on erythema						
1	1	1	1	1	2	2
2	2	*	2	2	*	2
3	0	0	0	0	0	0
4	0	0	0	0	1	1
5	0	1	2	0	2	2
6	2	2	1	2	1	1
7	0	0	0	0	0	0

* Patient withdrawn from the study because of a severe relapse.

Table IV. Expression of Ki-67 positive cells

patient	before treatment	13-cis-RA side	cream-base treated side
1	99	174	95
2	56	180	244
3	166	141	103
4	90	122	225
5	140	152	332
6	238	115	57
7	81	147	240
average	124	162	185

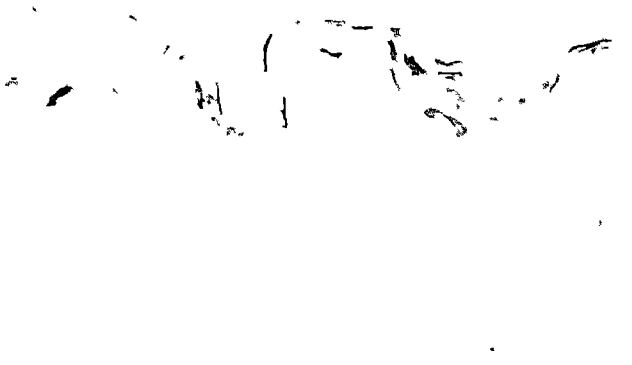

Fig. 1 Staining pattern of the monoclonal antibody 6B10, indicating keratin 4, in patient 3 at the 13-cis-RA treated side, after treatment.

DISCUSSION

Seven patients with Darier's disease were treated in a prospective, double-blind, bilaterally paired study, with a cream containing 13-cis-retinoic acid 0.1% and its cream-base only, for a period varying between 3 and 10 weeks. A statistically significant reduction of papules was observed. Irritation was minimal and could be controlled easily by reduction of the application frequency. Elongation of the treatment beyond the first month caused further improvement of the clinical results. Immunohistochemical assessment, revealed staining of keratin 4 in 3 and keratin 13 in 2 patients, at the 13-cis-RA treated side only. No induction was observed at baseline. Compared to baseline, Ki-67 was elevated after treatment at both sides. Nevertheless, the average number of Ki-67 positive cells at the 13-cis-RA treated side was lower than that at the cream-base treated side.

The good clinical results are in accordance with previous observations (7,8,9). Increasing the length of the treatment period from 4 to 10 weeks, improved the response to topical retinoids. Those patients not responding to topical 13-cis-RA, and requiring additional treatment, improved on systemic retinoids, indicating that oral acitretin is more effective. As keratin 4 was induced in 3/4 responders and keratin 13 in 1 responder and 1 non-responder, induction seems to offer an immunohistochemical tool for the investigation of the mode of action of 13-cis-RA, as suggested previously (8). The increase of the number of cycling cells after treatment showed great interindividual differences without a clear correlation with the clinical results, and therefore remains difficult to explain. However, the tendency for proliferation to be lower at the 13-cis-RA treated side, compared to the cream-base treated side, might be explained by the normalizing effect of retinoids on deregulated proliferation (11).

Topical treatment with retinoids may be improved by raising the concentration to 0.2%

(11) or even 0.3% (12). Addition of UVA-blocking agents may prevent conversion of isotretinoin to tretinoin (13), and therefore reduce local irritation, so the concentration of 13-cis-RA can be raised even more. Application of retinoids under plastic occlusion have been reported to induce remissions, persisting for about 3 months at the treated areas (4,5). The choice of the cream base is essential, for retinoid absorption is highly vehicle dependent. The penetration through stratum corneum and hence bioavailability of topical retinoids, is greatest when the drug is administered in isopropyl alcohol, followed by mineral oil, diisopropyl adipate and polyethylene glycol (14). Other retinoids may be more effective as a topical treatment for Darier's disease. With respect to interference with epidermal differentiation, arotinoids are the most potential retinoids, followed by etetrinate, tetrazol-retinamides and isotretinoin/tretinoin (15).

It has been shown that topical application of the retinoids isotretinoin and acitretin can produce dermal concentrations in excess of those produced by therapeutical oral doses of these drugs (14). Therefore it is attractive to speculate that topical treatment with retinoids constitutes a promising approach in the treatment of Darier's disease.

At the moment, however, oral retinoids seem to be more potent in the treatment of Darier's disease than the topical retinoids available. Nevertheless, topical 13-cis-RA 0.1% offers a new and well-tolerated alternative for systemic retinoid treatment in Darier's disease. Optimalisation of bioavailability and development of new topical retinoids are worthwhile.

ACKNOWLEDGEMENTS

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REFERENCES

1. Dicken CH, Bauer EA, Hazen PG, Krueger GG, Marks JG, Mc Guire JS, Schachner LA. Isotretinoin treatment of Darier's disease. J Am Acad Dermatol 1982;6:721-726.
2. Stüttgen G. Zur Lokalbehandlung von Keratosen mit Vitamin-A-Säure. Dermatologica 1962;124:65-80.
3. Beer P. Untersuchungen über die Wirkung der Vitamin-A-Säure. Dermatologica 1962;124:192-195.
4. Fulton JE, Gross PR, Cornelius CE, Kligman AM. Darier's disease. Treatment with topical vitamin A acid. Arch Derm 1968;98:396-399.
5. Plewig G, Wolff HH, Braun-Falco O. Lokalbehandlung normaler und pathologischer menschlicher Haut mit Vitamin-A-Säure. Klinische, histologische und elektronenmikroskopische Untersuchungen. Arch Klin Exp Dermatol 1971;239:390-413.
6. Muller SA, Belcher RW, Esterly NB, Lochner JC, Miller JS, Roenigk H, Weismann L. Keratinizing dermatoses, combined data from four centers on short-term topical treatment with tretinoin. Arch Dermatol 1977;113:1052-1054.
7. Steijlen PM, Happle R, van Muijen GNP, van de Kerkhof PCM. Topical treatment with 13-cis-

retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. Dermatologica 1991;182:178-183.

8. Steijlen PM, Reifenschweiler DOH, Ramaekers FCS, van Muijen GNP, Happle R, Link M, Ruiter DJ, van de Kerkhof PCM. Topical treatment of ichthyoses and Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study. Arch Dermatol Res 1993;285:221-226.
9. Burge SM, Buxton PK. Topical isotretinoin in Darier's disease. Br J Dermatol 1995;133:924-928.
10. Lucker GPH, van de Kerkhof PCM, Castelijns FACM, Steijlen PM. Topical treatment of ichthyoses with 13-cis-retinoic acid. A clinical and immunohistochemical study. Eur J Dermatol 1995;5:566-571.
11. Fritsch PO. Retinoids in psoriasis and disorders of keratinization. J Am Acad Dermatol 1992;27:S8-S14.
12. Plewig G, Ruhfus A, Klövekorn W. Sebum suppression after topical application of retinoids (arotinoid and isotretinoin). J Invest Dermatol 1983;80:357.
13. Elbaum DJ. Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne. J Am Acad Dermatol 1988;19:486-491.
14. Lehman PA, Slattery JT, Franz TJ. Percutaneous absorption of retinoids: influence of vehicle, light exposure and dose. J Invest Dermatol 1988;91:56-61.
15. Chung JC, Law MYL, Elliott ST, Elias PM. Diazacholesterol-induced ichthyosis in the hairless mouse. Assay for comparative potency of topical retinoids. Arch Dermatol 1984;120:342-347.

Palmoplantar keratodermas

Part 2

Introduction

As has been elucidated in the general introduction, the development of the nosology of HPPK's has not yet been completed so far. New reports of cases which do not fit in the existing nosology require a critical reappraisal of the classification. The absence or presence of associated clinical conditions, not fitting in the available descriptions, and therefore warranting extension of the previous nosology, are illustrated in chapters 1 and 2 of this part of the thesis. Chapter 3 demonstrates the simultaneous presence of two previously considered unrelated HPPK's, enabling a simplification of the present nosology. For day to day praxis, misdiagnosis of HPPK's may have a serious long-term impact on the quality of life of a patient. In chapter 4 the consequences of a misdiagnosis of a patient suffering from Olmsted's disease as acrodermatitis enteropatica who has been treated with zinc suppletion for years, illustrate such. Chapter 5 further illustrates that classifying the individual patient with a HPPK properly is not an academical exercise, but of practical relevance for good patient management. In this patient unfortunately, the correct diagnosis HPPK of Huriez was just made after she had developed 3 squamous cell carcinomas of the affected skin, which might have been prevented by a correct diagnosis in an earlier stage. Concerning therapeutical options, systemic retinoids might be effective for several HPPK's. Nevertheless, HPPK's exhibit distinct disease specificity concerning the sensitivity to retinoids (1). Good results have been reported in HPPK Greither (2), Papillon-Lefèvre (1, 3-5), mal de Meleda (1,6-8), Vohwinkel (9-11), and pachyonychia congenita (12,13). Mild improvement has been noticed in PPK varians Wachters (1), whereas variable results have been achieved in HPPK Unna-Thost (1) and the Olmsted's syndrome (14-17). In case of epidermolytic hyperkeratosis, retinoid treatment usually leads to severe skin erosions and consequently intolerable tenderness of the palmoplantar skin (18,19). Therefore, the search for alternatives for systemic retinoids is important, particularly in HPPK's poorly responding to retinoid treatment such as Vörner's disease. Chapter 4 introduces an alternative therapeutical method which proved to be successful in alleviating the pain in a case with HPPK of Olmsted, poorly responding to retinoids. In chapter 6 a topical treatment is described in HPPK of Vörner, which might offer an alternative to retinoid treatment.
Classification should be comprehensive, but simple. Classification should on one hand define separate diseases in a genetic sense, but on the other hand provide the dermatologist with leads to the management of care. Finally, in chapter 7 a new approach to the nosology of palmoplantar keratodermas is provided.

REFERENCES

1. Blanchet-Bardon C, Nazarro V, Rognin C, Geiger JM, Puissant A. Acitretin in the treatment of severe disorders of keratinization. Results of an open study. J Am Acad Dermatol 1991;24:982-986.

2. van de Kerkhof PCM, van Dooren-Greebe RJ, Steijlen PM. On the efficacy of acitretin in keratodermia palmaris et plantaris transgrediens et progrediens Greither. Eur J Dermatol 1992;2:503-505.
3. Bergman R, Friedman-Birnbaum R. Papillon-Lefèvre syndrome; a study of the long-term clinical course of recurrent pyogenic infections and the effects of etretinate treatment. Br J Dermatol 1988;119:731-736.
4. Wehrmann W, Traupe H, Happle R. Papillon-Lefèvre syndrome (keratosis palmoplantaris mit periodontopathie); Behandlung mit etretinate. Hautarzt 1985;36:173-175.
5. Nazarro V, Blanchet-Bardon C, Mimoz C, Revuz J, Puissant A. Papillon-Lefèvre syndrome; ultrastructural study and successful treatment with acitretin. Arch Dermatol 1988;124:533-539.
6. Jee SSH, Lee YY, Wu YC, Lu YC, Pan CC. Report of a family with mal de Meleda in Taiwan: A clinical, histopathological and immunological study. Dermatologica 1985;171:30-37.
7. van de Kerkhof PCM, van Dooren-Greebe RJ, Steijlen PM. Acitretin in the treatment of mal de Meleda. Br J Dermatol 1992;127:191-192.
8. Brambilla L, Pigatto PD, Boneschi V. Unusual cases of Meleda keratoderma treated with aromatic retinoid etretinate. Dermatologica 1984;168:283-286.
9. Chang Sing Pang AFI, Oranje AP, Vuzevki VD. Successful treatment of keratoderma hereditaria mutilans with an aromatic retinoid. Arch Dermatol 1981;117:225-228.
10. Camisa C, Rossana C. Variant of keratoderma hereditaria mutilans (Vohwinkel's syndrome): treatment with orally administered isotretinoin. Arch Dermatol 1984;120:1323-1328.
11. Rivers JK, Duke EE, Justus DW. Etretinate: management of keratoderma hereditaria mutilans in four family members. J Am Acad Dermatol 1985;13:43-49.
12. Dupré A, Christol B, Bonafé JL, Touron P. Pachyonychie congénitale. Description de 3 cas familiaux. Traitement par le rétinoïde aromatique (RO 10.9359). Ann Dermatol Venereol 1981;108:145-149.
13. Thomas DR, Jorizzo JL, Brysk MM. Pachyonychia congenita. Electron microscopic and epidermal glycoprotein assessment before and during isotretinoin treatment. Arch Dermatol 1984;120:1475-1479.
14. Poulin Y, Perry HO, Muller SA. Olmsted syndrome - congenital palmoplantar and periorificial keratoderma. J Am Acad Dermatol 1984;10:600-10.
15. Atherton DJ, Sutton C, Jones BM. Mutilating palmoplantar keratoderma with periorificial keratotic plaques. Br J Dermatol 1990;122:245-252.
16. Judge MR, Misch K, Wright P, Harper JI. Palmoplantar and periorificial keratoderma with corneal epithelial dysplasia: a new syndrome. Br J Dermatol 1991;125:186-188.
17. Haußer I, Frantzmann Y, Anton-Lamprecht I, Estes S, Frosch PJ. Olmsted Syndrom. Erfolgreiche Therapie durch Behandlung mit Etretinat. Hautarzt 1993;44:394-400.
18. Fritsch P, Hönigsmann H, Jaschke E. Epidermolytic palmoplantar keratoderma. Br J Dermatol 1978;99:561-568.
19. Requena L, Schoendorff C, Sanchez Yus E. Hereditary epidermolytic palmoplantar keratoderma (Vörner-type). Report of a family and review of the literature. Clin Exp Dermatol 1991;16:383-388.

Chapter 1

Pachyonychia congenita in the absence of other syndrome abnormalities.

A Chang
GPH Lucker
PCM van de Kerkhof
PM Steijlen

J Am Acad Dermatol 1994;30:1017-1018

INTRODUCTION

Pachyonychia congenita was first described by Jadassohn and Lewandowski [1] in 1906. Nail changes that affect all nails symmetrically are the hallmark of the disease. The base of the nails appears to be normal but the free edge is raised by a thick, subungual hyperkeratosis. The lateral borders often angulate toward the center. Associated symptoms include palmoplantar hyperkeratosis, follicular keratosis, xerodermia, palmoplantar hyperhidrosis, and blisters on the soles of the feet. Leukokeratosis of the tongue and buccal mucosa, corneal abnormalities, and malformations of the teeth have also been described [2]. On the basis of the presence and prevalence of associated symptoms, numerous subdivisions of pachyonychia congenita have been suggested [3-5]. Both autosomal dominant and autosomal recessive [6] forms have been described, reflecting heterogeneity.

We describe a Moroccan family with nail deformities in the abscence of other syndrome abnormalities, which demonstrates that the coexistence of associated symptoms is not an absolute prerequisite for the diagnosis of pachyonychia congenita.

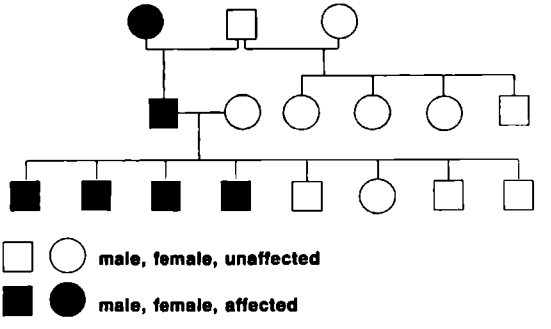


Fig. 1 Pedigree of family manifesting a monosymptomatic form of pachyonychia congenita. Circle, Female; square, male; shaded symbols, affected; open symbols, unaffected.

CASE REPORT

Several members of a family had nail deformities that had been present since the first months of life. Affected members were the grandmother, the father, and his four sons, ages 73, 51, 21, 19, 16, and 13 years, respectively. The mother and four other siblings were not affected (Fig. 1). There was no history of consanguinity. In the affected persons

all nails of hands and feet showed symmetric thickening and hardening, with yellow-brown discoloration, subungual hyperkeratosis, and upward growth of the distal nail with hypercurvature (Fig. 2). On further examination, the affected persons had no deformities of the skin, hair, mucous membranes, eyes, or teeth. To exclude onychomycosis, nail cultures were examined for the presence of fungi. All cultures were negative.
No treatment was given because the patients' complaints were of a cosmetic nature only.

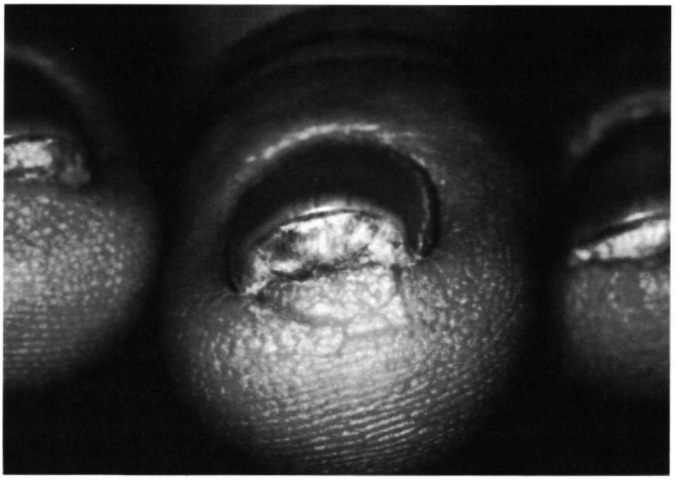


Fig. 2 Fingernail shows thickened nail plate, subungual hyperkeratosis, and upward growth of the distal nail with hypercurvature.

DISCUSSION

The nail changes of all five affected family members consisted of symmetric thickening, subungual hyperkeratosis, and upward growth of the distal nail with angulation of the lateral borders toward the center. All nails were equally affected. The nail changes had been present since the first months of life. The pedigree is consistent with an autosomal dominant mode of inheritance.
In the differential diagnosis, congenital onychogryphosis and pachyonychia congenita must be considered. Congenital onychogryphosis is a hereditary nail disorder with an autosomal dominant mode of inheritance. Associated features are lacking. Onychogryphosis appears in adolescence, affects predominantly the thumb and large toenails, and lacks massive subungual hyperkeratosis; the nails curve downward at the free margin [7]. Pachyonychia congenita is characterized by nail changes similar to those of our patients; all nails are symmetrically affected, and the condition appears within the

first months of life. Furthermore, the coexistence of associated features is characteristic of the disease.

The nail deformities present in our patients are characteristic of pachyonychia congenita, although the associated findings of the syndrome are lacking. Therefore the family appears to represent a monosymptomatic form of pachyonychia congenita. In thse patients the same gene is probably affected as in other cases of autosomal dominant pachyonychia congenita; however, it may represent a different allele, resulting in a milder phenotype.

REFERENCES

1. Jadassohn J, Lewandowski F. Pachyonychia congenita. Keratosis disseminata circumscripta (follicularis). Tylomata. Leukokeratosis linguae. vol 1. In: Jacobs Ikonographia Dermatologica. Berlin: Urban and Schwarzenberg 1906:29-31.
2. Feinstein F, Friedman J, Schewach-Millet M. Pachyonychia congenita. J Am Acad Dermatol 1988;19:705-11.
3. Kumer L, Loos Ho. congenital pachyonychia (Riehl type). Wien Klin Wochenschr 1935;48:174-8.
4. Schonfeld PHIR. The pachyonychia congenita syndrome. Acta Derm Venereol (Stockh) 1980;60:45-9.
5. Sivasundram A, Rajagopalan K, Sarojini T. Pachyonychia congenita. Int J Dermatol 1985;24:179-80.
6. Haber RM, Rose TH. Autosomal recessive pachyonychia congenita. Arch Dermatol 1986;122:919-23.
7. Lubach D. Erbliche Onychogryposis. Hautarzt 1982;33:331-334.

Chapter 2

Pachyonychia congenita tarda.

GPH Lucker
PM Steijlen

Clin Exp Dermatol 1995;20:226-229

SUMMARY

Pachyonychia congenita is a distinct hereditary disorder of keratinization, in which dystrophy of all nails is associated with palmoplantar keratoderma and other hyperkeratoses. Recently a late-onset type has been reported. We report a second family with late-onset pachyonychia congenita, showing a remarkable clinical heterogeneity. Furthermore, one patient demonstrated a number of associated hyperkeratoses not previously recognized. Acitretin proved useful in the treatment of this late-onset form of pachyonychia congenita.

INTRODUCTION

Pachyonychia congenita has been described by Muller in 1904 [1] and Wilson in 1905 [2]. Jadassohn and Lewandowski reported in 1906 on the association with palmoplantar keratoderma and other ectodermal defects [3]. Since then, the presence of associated features has been considered essential for the diagnosis. Based on the prevalence of the associated symptoms, several classifications have been proposed [4-7]. We recently described a monosymptomatic form of the disease, lacking the associated anomalies of the syndrome [8]. Both autosomal dominant and autosomal recessive [9] modes of inheritance have been described, reflecting genetic heterogeneity. Dystrophy of all nails is the main feature of the syndrome and usually presents within the first months of life.

Recently five patients were observed with the onset of the characteristic nail changes during the second and third decades of life [10]. The term pachyonychia congenita tarda was proposed to describe this subset of pachyonychia congenita, characterized by palmoplantar keratoderma, hyperhidrosis and oral leucokeratosis associated with the nail lesions. No other hyperkeratoses were found to be associated.

We have observed a second family with the late-onset type of pachyonychia congenita, exhibiting remarkable phenotypic heterogeneity. Combined topical keratolytic and oral retinoid treatment was started in one family member with a number of associated hyperkeratoses.

CASE REPORT

A 45-year-old man presented with a hyperkeratotic skin disorder localized to the nails, palms, soles, knees, lower legs, face and scalp. The dermatosis had started at the age of 44 and had progressed rapidly. Painful plantar keratoderma prevented him from walking.

On examination, all nails were dystrophic (Fig. 1). The free edges were raised by thick subungual hyperkeratosis with angulation of the lateral borders towards the centre. All nails appeared to be affected equally. In addition, a diffuse, thick, warty palmoplantar keratoderma was present (Figs 2 and 3). Nummular hyperkeratoses were observed at the knees, and similar lesions were noticed at the forehead and the scalp. The lower legs were covered by large brown scales, mimicking lamellar ichthyosis.

Family history revealed that his brother and father had similar, although less pronounced

nail changes (Fig 4), which had started in, respectively, the third and fourth decades of age. Neither had additional hyperkeratoses.

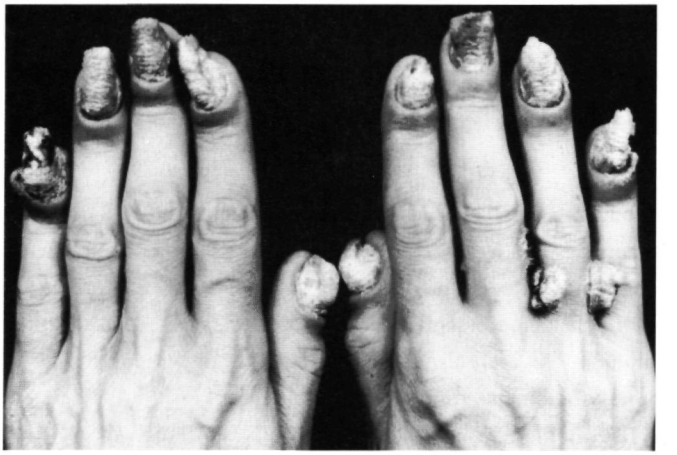


Fig. 1 Characteristic dystrophic changes, affecting all nails symmetrically. At the dorsum of the right hand, two verrucous hyperkeratotic plaques are visible, extending from the palmar skin to the dorsal surfaces.

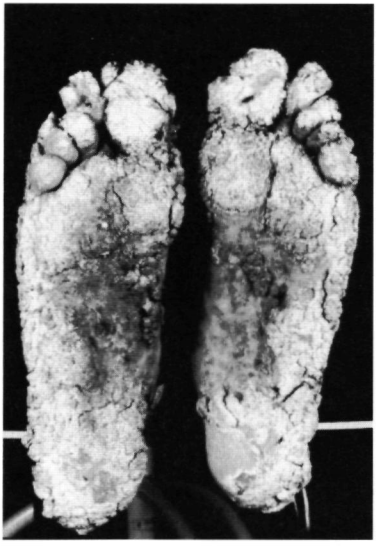


Fig. 2 The plantar skin is diffusely covered by a thick, warty hyperkeratosis, causing painful fissures.

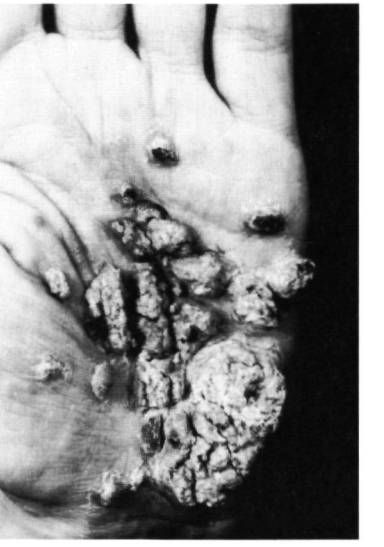


Fig. 3 Verrucous hyperkeratotic plaques at the palmar surfaces.

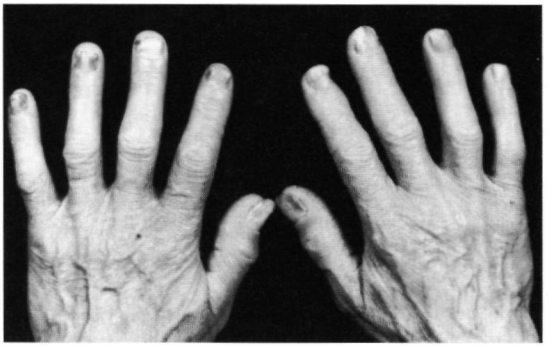


Fig. 4 Nail changes observed in the patient's father, less pronounced though similar to those presented in Figure 1.

Histopathological examination of a biopsy taken from the palmar skin revealed hyperkeratosis and acanthosis, consistent with palmoplantar keratoderma. Mycological culture and microscopic examination of the subungual hyperkeratosis proved negative.
To remove the hyperkeratoses, topical treatment was given with salicylic acid 20% in petrolatum, resulting in a significant improvement within three weeks. Subsequently, oral retinoid treatment was started with acitretin 35 mg daily, to prevent new hyperkeratoses from developing. After 2.5 weeks of therapy, a further reduction of hyperkeratoses was observed (Fig. 5) and the daily dosage of acitretin was reduced to 20 mg for the next 6 weeks, with continued improvement. Because of elevated liver enzymes, the daily dosage was further reduced to 10 mg for 4 weeks and eventually stopped, resulting in a relapse of the hyperkeratoses. The nail changes, however, responded poorly to the retinoid therapy.

DISCUSSION

The present communication reports a second family with pachyonychia congenita tarda. The characteristic nail changes, which had started in the third and fourth decades of life, were observed in all three affected family members. The patient described exhibited, in addition, a diffuse, incapacitating palmoplantar keratoderma, ichthyosiform scaling on both legs and nummular hyperkeratoses at the knees, face and scalp, which appeared simultaneously with the nail dystrophy. Associated dyskeratoses were absent in his two affected family members.
In classical pachyonychia congenita, however, hyperkeratotic skin lesions, such as palmoplantar keratoderma, follicular keratosis, verrucosities over knees, elbows, buttocks and popliteal area, and oral leucokeratosis, have been well documented to be variably associated [11]. The range of associated hyperkeratoses and the variable expression we observed in our case with the late-onset variant of pachyonychia congenita are, therefore,

similar to pachyonychia congenita. Nevertheless, some additional hyperkeratoses we observed, including nummular hyperkeratoses on the face and scalp, as well as the ichthyosiform scaling on both legs, have not been described previously and are therefore new to the syndrome.

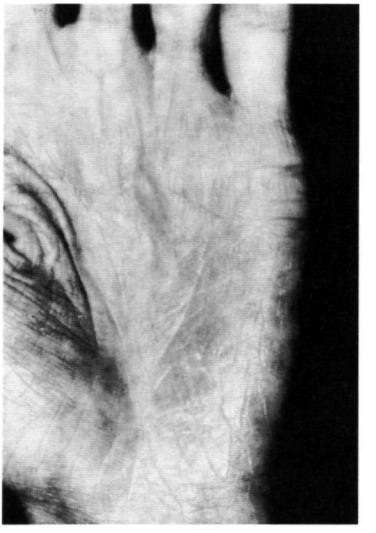


Fig. 5 Palmar skin after 3 weeks of local keratolytic and 2.5 weeks of acitretin treatment.

The inheritance pattern of our pedigree is highly suggestive of an autosomal dominant mode of inheritance, and therefore consistent with the other family with pachyonychia congenita tarda described [10].

There are no reports available at the moment on the therapy of pachyonychia congenita tarda. In classical pachyonychia congenita, retinoids have been reported to be effective in alleviating the palmoplantar keratoderma, the subungual hyperkeratosis [12], the keratotic patches on the knees and the leucokeratotic lesions in the mouth [13]. One of our cases demonstrates acitretin to be effective in preventing new hyperkeratoses from developing in the late-onset type. Simple local keratolytic measures proved useful in reducing the hyperkeratoses.

We conclude that both the classical and late-onset variant of pachyonychia congenita are characterized by heterogeneity in expression of a number of associated hyperkeratoses. It seems rational to distinguish pachyonychia congenita tarda from pachyonychia congenita by the differences in age of onset. Retinoids constitute a promising approach to the treatment of the hyperkeratoses in both variants.

REFERENCES

1. Muller C. On the causes of congenital onychogryphosis. München Med Wchnschr 1904;49:2180-2.
2. Wilson AG. Three cases of hereditary hyperkeratosis of the nail bed. Br J Dermatol 1905;17:13-4.
3. Jadassohn J, Lewandowski F. Pachyonychia congenita. Keratosis disseminata circumscripta (follicularis). Tylomata. Leukokeratosis linguae. In: Neisser A, Jacobi E eds. Ikonographia Dermatologica, Vol.1. Berlin: Urban and Schwarzenberg, 1906:29-31.
4. Kumer L, Loos HO. Congenital pachyonychia (Riehl type). Wien Klin Wochenschr 1935;48:174-8.
5. Schönfeld PHIR. The pachyonychia congenita syndrome. Acta Derm Venereol (Stockh) 1980;60:45-9.
6. Sivasundram A, Rajagopalan K, Sarojini T. Pachyonychia congenita. Int J Dermatol 1985;24:179-80.
7. Feinstein A, Friedman J, Schewach-Millet M. Pachyonychia congenita. J Am Acad Dermatol 1988;19:705-11.
8. Chang A, Lucker GPH, Kerkhof PCM van de, Steijlen PM. Pachyonychia congenita in the abscence of other syndrome abnormalities. J Am Acad Dermatol 1994;30:1017-8.
9. Haber RM, Rose TH. Autosomal recessive pachyonychia congenita. J Am Acad Dermatol 1988;19:705-11.
10. Paller AS, Moore JA, Scher R. Pachyonychia congenita tarda. A late-onset form of pachyonychia congenita. Arch Dermatol 1991;127:701-703.
11. Poulin Y, Perry HO, Muller SA. Olmsted syndrome - congenital palmoplantar and periorificial keratoderma. J Am Acad Dermatol 1984;10:600-10.
12. Dupré A, Christol B, Bonafé JL, Touron P. Pachyonychie congénitale. Description de 3 cas familiaux . Traitement par le rétinoïde aromatique (RO 10.9359). Ann Dermatol Venereol 1981;108:145-149.
13. Thomas DR, Jorizzo JL, Brysk MM, Tschen JA, Miller J, Tschen EH. Pachyonychia congenita. Electron microscopic and epidermal glycoprotein assessment before and during isotretinoin treatment. Arch Dermatol 1984:120:1475-1479.

Chapter 3

Keratosis palmoplantaris varians et punctata. Klinische Variabilität eines einzigen genetischen Defektes?.

GPH Lucker
PM Steijlen

Hautarzt, accepted

ZUSAMMENFASSUNG

Keratosis palmoplantaris (KPP) varians und KPP punctata werden bisher als unterschiedliche Formen der kongenitalen Palmoplantarkeratosen aufgefaßt. Die Keratosis punctata der Palmarfurchen, durch stecknadelkopfgroße Hyperkeratosen innerhalb der palmaren Falten charakterisiert, ist als eigenständige Erscheinungsform einer KPP beschrieben worden. Wir berichten über einen 31jährigen Mann mit sowohl inselförmigen als auch erbsengroßen Hyperkeratosen an den Fußsohlen sowie stecknadelkopfgroßen Hyperkeratosen in den Palmarfurchen. Aufgrund der Koexistenz dieser kongenitalen Hautveränderungen kann man sich die Frage stellen ob KPP varians und KPP punctata wirklich eigenständige Entitäten sind oder variable Manifestationen ein und desselben Gendefekts darstellen.

EINLEITUNG

Verhornungsstörungen der Handteller und Fußsohlen stellen ein dermatologisches Leitsymptom für zahlreiche erbliche und nichterbliche Krankheitsbilder dar. Aufgrund morphologischer, histologischer und genetischer Kriterien können die hereditären Palmoplantarkeratosen im engeren Sinne klassifiziert werden, wobei gesetzmäßig auftretende assoziierte Symptome für eine weitere Differenzierung herangezogen werden [5]. Inselförmige Palmoplantarkeratosen ohne assoziierte Symptome werden als "Keratosis palmoplantaris varians" bezeichnet, wobei jedoch auch streifenförmige Formen einbezogen werden [6]. Histologisch läßt sich hiervon die klinisch ähnliche Keratosis palmoplantaris nummularis unterscheiden, da diese durch eine epidermolytische Hyperkeratose kennzeichnet ist [7]. Punktförmige Palmoplantarkeratosen ohne assoziierte Symptome werden in Keratosis palmoplantaris punctata [2], Akrokeratoelastoidose [3] und fokale akrale Hyperkeratose [1] unterteilt. Die Keratosis punctata der Palmarfurchen [8], als Variante der Keratosis palmoplantaris punctata beschrieben, ist durch kleine Hyperkeratosen in den Palmarfurchen charakterisiert.

Berichtet wird über einen 31jährigen Patienten, der sich mit insel- und punktförmigen Hyperkeratosen an den Fußsohlen und vereinzelten punktförmigen Hyperkeratosen in den Beugefalten der Handflächen vorstellte. Ein zusammentreffen dieser bisher als eigenständig geltenden Entitäten ist, soweit uns bekannt, bislang noch nicht beschrieben worden.

FALLBERICHT

Ein 31jähriger Mann stellte sich mit schmerzhaften, symmetrischen Hyperkeratosen an den Fußsohlen vor. Bei der klinischen Untersuchung wurden sowohl inselförmige als auch erbsgroße Hyperkeratosen an beiden Fußsohlen beobachtet (Abb. 1). Stecknadelkopfgroße hyperkeratotische Papeln waren in den Beugefalten der Handflächen lokalisiert (Abb. 2). Assoziierte Symptome fehlten.

Die Hyperkeratosen hatten sich erstmalig im Alter von 14 Jahren entwickelt. Nach

Exzision einiger Hyperkeratosen an den Fußsohlen zum Zwecke der Schmerzlinderung, traten innerhalb von 3 Wochen Rezidive auf.

Bei der Mutter des Patienten bestanden ähnliche Hautveränderungen.

Die histologische Untersuchung einer Biopsie von der Fußsohle ergab eine ausgeprägte Hyperkeratose mit Parakeratose und Akanthose. Es bestand keine epidermolytische Hyperkeratose.

Die hämatologischen Laborbefunde waren normal.

Eine Therapie mit 30%iger Salizylvaseline führte zu einer Linderung der Hyperkeratosen.

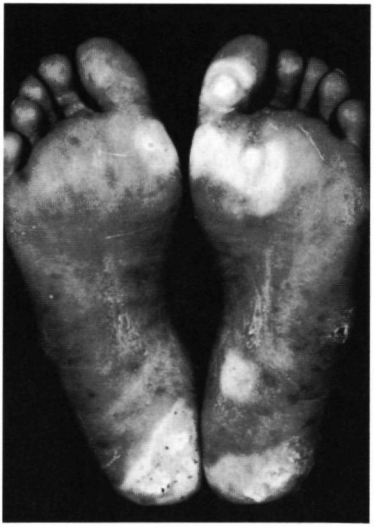


Abb 1. Grosse inselförmige und erbsengroße Hyperkeratosen an den Fußsohlen. Zustand nach zweiwöchiger keratolytischer Behandlung.

Abb 2. Stecknadelkopfgroße Hyperkeratosen in den Palmarfurchen.

BESPRECHUNG

Die große klinisch-morphologische Vielfalt der hereditären Palmoplantarkeratosen, die uneinheitliche Nomenklatur und die Vielzahl kasuistischer Mitteilungen erschweren die Klassifikation. In den letzten Jahrzehnten sind nicht nur neue Typen beschrieben, sondern auch zuvor als eigenständig geltenden Typen zusammengefügt worden. So hat Küster nachgewiesen, daß die Keratosis palmoplantaris diffusa (Unna-Thost) histologisch durch epidermolytische Hyperkeratose charakterisiert und mit der Keratosis palmoplantaris cum degeneratione granulosa (Vörner) offenbar identisch ist [4]. Wachters' Untersuchungen haben ergeben daß die Keratosis palmoplantaris linearis-striata (Brünauer-Fuhs) und die Keratosis palmoplantaris areata (Siemens) unterschiedliche Manifestationen ein und derselben Genodermatose darstellen, und somit keine eigenständigen Krankheitsbilder sind [6].

Aufgrund unserer kasuistischer Mitteilung stellt sich die Frage ob KPP punctata und KPP varians eigenständige Entitäten sind. In diesem Fall würde unserer Patient an zwei verschiedenen Genodermatosen leiden. Wahrscheinlicher ist aber, daß es sich um einen einzigen Genotyp mit variabler phänotypischer Ausprägung handelt.

Man könnte annehmen, daß die KPP punctata und die KPP varians wahrscheinlich keine unabhängigen Krankheitsbilder, sondern variable klinische Erscheinungsforme einer einzigen Genodermatose darstellen.

LITERATUR

1. Dowd PM, Harman RRM, Black MM. Focal acral hyperkeratosis. Br J Dermatol 1983;109:97-103.
2. Friedman SJ, Herman PS, Pittelkow MR, Daniel WP. Punctate porokeratotic keratoderma. Arch Dermatol 1988;124:1678-1682.
3. Jung EG, Beil FU, Anton-Lamprecht I, Greten H, Nemetschek T. Akrokeratoelastoidosis. Hautarzt 1974;25:127-133.
4. Küster W, Becker A. Indication for the identity of palmoplantar keratoderma type Unna-Thost with Type Vörner. Thost's family revisited 110 years later. Acta Derm Venereol (Stockh) 1992;72:120-122.
5. Lucker GPH, van de Kerkhof PCM, Steijlen PM. The hereditary palmoplantar keratoses: an updated review and classification. Br J Dermatol 1994;131:1-14.
6. Wachters DHJ. Over de verschillende morphologische vormen van de keratosis palmoplantaris, in het bijzonder over de "keratosis palmoplantaris varians". Thesis, Leiden, The Netherlands 1963:49-67.
7. Wachters DHJ, Frensdorf EL, Hausman R, van Dijk E. Keratosis palmoplantaris nummularis ("hereditary painful callosities"). J Am Acad Dermatol 1983;9:204-209.
8. Weiss RM, Rasmussen JE. Keratosis punctata of the palmar creases. Arch Dermatol 1980;116:669-671.

Chapter 4

The Olmsted syndrome: Mutilating palmoplantar and periorificial keratoderma.

GPH Lucker
PM Steijlen

J Am Acad Dermatol 1994;31:508-509

INTRODUCTION

In 1927, Olmsted [1] described a congenital palmoplantar keratoderma characterized by sharply marginated, diffuse, warty hyperkeratosis of the palms and soles, leading to flexural deformities and spontaneous amputation of two terminal phalanges. A pronounced subungual and circumungual hyperkeratosis also occurs, curving the nails laterally and anterioposteriorly. Hyperkeratotic plaques parallel to the mucocutaneous junction of the lower lip and at the angles of the mouth are also present. Similar lesions occur around the umbilicus and the anus.

To date eight cases have been reported [1-8]. Transmission from mother to son has been observed [3,7], which suggests an autosomal dominant mode of inheritance. Local measures such as repeated prolonged soaking of the affected parts in warm water followed by mechanical removal of the thickened epidermis [1], full thickness excision of the palmar skin, followed by skin grafting [7] as well as oral etretinate [8] have been reported to be effective in single cases.

We describe a patient with the typical characteristics of the syndrome, who was treated with full thickness excision of the palmar and plantar skin, followed by skin grafting.

CASE REPORT

A 33-year-old man had a hyperkeratotic disorder, which appeared on the palmoplantar skin when he was 3 months old. The palmoplantar keratoderma gradually progressed to a thick, warty hyperkeratosis, diffusely covering the palms and soles, and leading to painful flexion contractures of the fingers and toes. A family history of similar lesions was lacking. The skin disorder had previously been diagnosed as acrodermatitis enteropathica, although the plasma zinc concentration was normal. Treatment with oral zinc sulfate for several years did not cause significant improvement. Strict relief of external factors resulted in a much slower development of new lesions, although this could not prevent hyperkeratoses from developing. Hyperkeratosis gradually recurred focally, necessitating surgical removal and transplantation annually.

In addition to the palmar and plantar hyperkeratoses (Fig. 1), examination revealed prominent nail dystrophy and perioral, perinasal (Fig. 2), and perianal hyperkeratoses. A universal alopecia was also present.

Histopathological examination of the palmar skin displayed massive hyperkeratosis and a reduced to absent granular cell layer. In the thickened horny layer, many cells showed parakeratosis and perinuclear edema.

Because the flexion contractures were extremely painful, a full-thickness excision of the palmar skin, followed by skin grafting plus arthrolysis and arthrodesis of the finger joints, were performed, before referral to our clinic. Although the joint contractures failed to improve, a substantial relief of pain was achieved, and the plantar skin was similarly treated. In addition, amputation of the toes was performed at the patient's request, to achieve optimal pain reduction. Because the patient noted a striking relation

between trauma and exacerbation of the hyperkeratosis, he decided to avoid pressure on the soles, by using a wheelchair or creeping. Subsequently, hyperkeratoses developed on both knees. After referral to our dermatology department, treatment with the oral retinoid acitretin was proposed to prevent new hyperkeratoses from developing. However, this option was rejected by the patient because his pain was limited and he feared possible side effects.

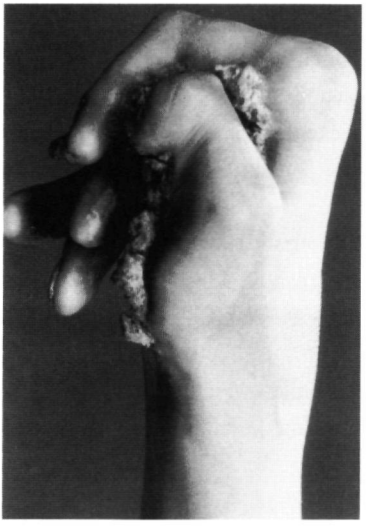


Fig. 1 Before treatment, warty hyperkeratosis diffusely covers palmar sites of both hands and fingers. Keratoderma does not involve dorsal surfaces. Painful flexion contractures are prominent. Most fingernails are dystrophic.

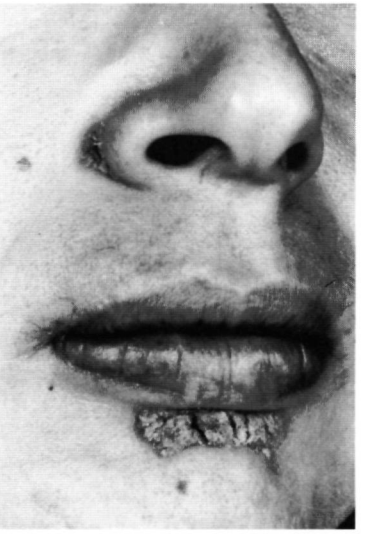


Fig. 2 Hyperkeratotic plaques beneath lower lip and in nasolabial fold.

DISCUSSION

We have described a patient with typical Olmsted syndrome who also had universal alopecia and hyperkeratotic plaques on both knees. A striking relation was noted between trauma and the hyperkeratoses. In the differential diagnosis, Clouston's syndrome, acrodermatitis enteropathica, keratoderma mutilans of Vohwinkel, mal de Meleda, and pachyonychia congenita have to be considered. Clouston's syndrome is characterized by alopecia and a nonmutilating palmoplantar keratoderma. This can usually be differentiated easily from Olmsted syndrome by the absence of periorificial keratoses. Acrodermatitis enteropathica mimics Olmsted syndrome and is caused by zinc deficiency. Our patient had been misdiagnosed as such and treated for years with zinc. Probably other cases have been similarly misdiagnosed.

Oral retinoids have proved effective in the treatment of several palmoplantar keratodermas [9], such as mutilating mal de Meleda [10] and palmoplantar keratoderma of Vohwinkel [11-13], but some failed to respond. One patient with Olmsted syndrome rapidly improved with etretinate, 0.5 mg/kg/day [8]. However, another patient failed to improve with etretinate, 1 mg/kg/day [7]. For the nonresponding patient, full-thickness excision followed by skin grafting, is an alternative to alleviate the pain.

REFERENCES

1. Olmsted HC. Keratodermia palmaris et plantaris congenitalis. Report of a case showing associated lesions of unusual location. Am J Dis Child 1927;33:757-764.
2. Costa OG. In: Acrokeratoses. University of Minas Geraes, Brazil: Belo Horizonte, 1962.
3. Keir M. Keratodermia palmaris et plantaris. J Roy Soc Med 1967;79:419-421.
4. Ruiz-Maldonado R, Lozano-Ferral N. Hiperqueratosis palmoplantar mutilante con hipotriquia y co lesiones eritematoescamosas inguinales y perianales. Int J Dermatol 1972;11:31-35.
5. Poulin Y, Perry HO, Muller SA. Olmsted syndrome - congenital palmoplantar and periorificial keratoderma. J Am Acad Dermatol 1984;10:600-10.
6. Harms M, Bergues JP, Saurat JH. Syndrome de Olmsted (kératodermie palmoplantaire et périorificielle congénitale). Ann Derm Venereol 1985;112:479.
7. Atherton DJ, Sutton C, Jones BM. Mutilating palmoplantar keratoderma with periorificial keratotic plaques. Br J Dermatol 1990;122:245-252.
8. Judge MR, Misch K, Wright P, Harper JI. Palmoplantar and periorificial keratoderma with corneal epithelial dysplasia: a new syndrome. Br J Dermatol 1991;125:186-188.
9. Blanchet-Bardon C, Nazzaro V, Rognin C, Geiger J, Puissant A. Acitretin in the treatment of severe disorders of keratinization. Results of an open study. J Am Acad Dermatol 1991;24:982-986.
10. Brambilla L, Pigatto PD, Boneschi V, Altomare GF, Finzi AF. Unusual cases of Meleda keratoderma treated with aromatic retinoid etretinate. Dermatologica 1984;168:283-286.
11. Chang Sing Pang AFI, Oranje AP, Vuzevki VD, Stolz E. Successful treatment of keratoderma hereditaria mutilans with an aromatic retinoid. Arch Dermatol 1981;117:225-228.
12. Camisa C, Rossana C. Variant of keratoderma hereditaria mutilans (Vohwinkel's syndrome). Treatment with orally administered isotretinoin. Arch Dermatol 1984;120:1323-1328.

13. Rivers JK, Duke EE, Justus DW. Etretinate: Management of keratoma hereditaria mutilans in four family members. J Am Acad dermatol 1985;13:43-49.

Chapter 5

The Huriez syndrome; Palmoplantar keratoderma with sclerodactyly.

GPH Lucker
N Zeedijk
PM Steijlen

Submitted

INTRODUCTION

In 1963, Huriez et al. described an autosomal dominantly inherited syndrome, characterized by a diffuse palmoplantar keratoderma with sclerodactyly and nail changes, in two families resident in the north of France (1-3). The palmoplantar keratoderma consists of a discrete, sometimes lamellated hyperkeratosis with atrophy, diffusely covering especially the palmar skin. The plantar skin usually displays less severe involvement. Atrophic plaques may be found on the dorsa of the hands and fingers. Obligatorily associated features are sclerodactyly and nail changes. The sclerodactyly strongly resembles sclerodermia, although the genetic inheritance, absence of systemic symptoms, lack of vasomotor phenomena, appearance of the initial symptoms at birth or in early childhood, and absence of progression during adulthood, enable a differentiation. Nail changes consist of aplasia, ridging and clubbing. Hypohidrosis is associated in half of the cases. The disease usually starts at birth or during early childhood and persists unchanged throughout adult life. The distinctive feature of this syndrome is the risk of the development of squamous cell carcinoma on the affected skin, which may occur as early as the third or fourth decade. Until now, seven families (1-8) and one case report (9) have been published. We describe another familial case affected with the Huriez syndrome.

CASE REPORT

A 34-year old woman presented with a palmoplantar keratoderma, focally involving the dorsal surfaces, that had appeared at the age of 1 year. At the age of 31, four hyperkeratotic nodules and plaques had developed progressively on the affected skin at the dorsal side of the left hand. After surgical removal, histopathological examination revealed squamous cell carcinoma in three of them.

Similar, though less pronounced palmoplantar hyperkeratosis, had appeared shortly after birth in her son. A family history of similar lesions was lacking. The maternal family history revealed that ancestors had lived in the north of France.

Examination of the mother revealed thin hyperkeratoses with erythema and atrophy on the palmoplantar skin, with small areas of normal skin in between (Fig. 1). The palmar lesions were restricted to the palms, and did not spread to the dorsal skin. In addition, irregular areas with similar lesions were observed at the dorsal skin of the hands. The dorsal skin of the feet displayed no abnormalities. All fingers of both hands revealed a marked sclerosis of the skin (Fig. 2). Most fingernails showed longitudinal ridging. Examination of her son revealed palmar erythema with slight hyperkeratotic desquamation. Erythematous, slightly hyperkeratotic plaques were observed on the dorsal skin overlying the finger joints of the hands (Fig. 3). Sclerodactyly prevented full extension of the fingers.

Histopathological examination of the palmar skin of the mother displayed hyperkeratosis, acanthosis and papillomatosis. Epidermolytic hyperkeratosis was absent.

Therapy was started with calcipotriol ointment once daily on the right palmar and plantar skin. After 5 weeks of therapy, no beneficial effect was observed. The application frequency of calcipotriol was doubled. In addition, oral acitretin treatment was started with a daily dose of 25mg. Mucocutaneous retinoid side effects were observed though not severe, and well tolerated. After 2 months of treatment, a slight reduction of the hyperkeratosis was observed. A left-right difference was absent.

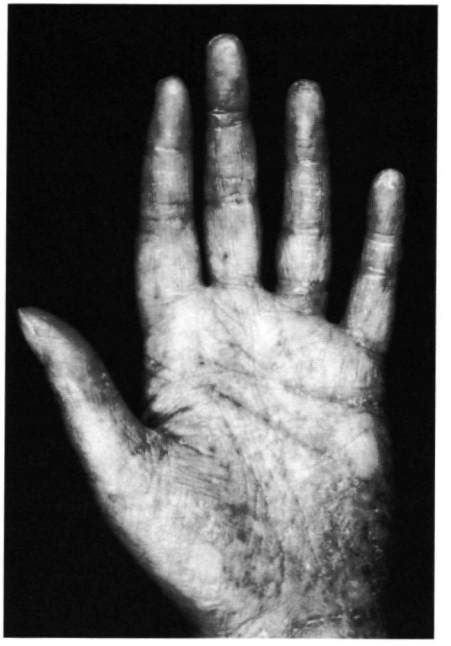


Fig. 1 Palmar skin of the mother, showing a diffuse, thin hyperkeratosis with erythema and atrophy. Dermatoglyphic patterns are lost. Extension of the fingers is limited due to sclerodactyly.

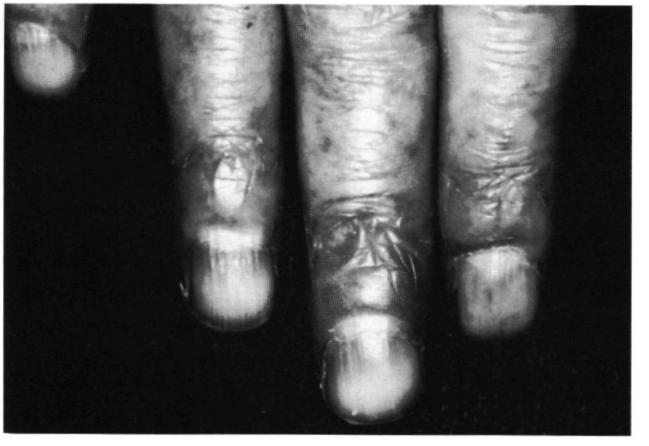


Fig. 2 Sclerosis of the skin of all fingers. The fingernails display ridging.

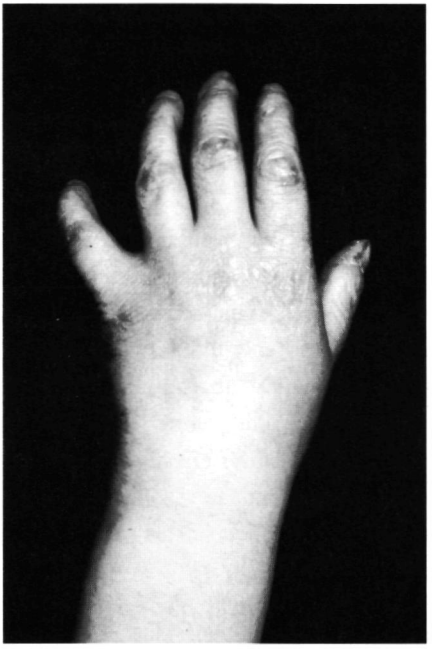


Fig. 3 Atrophic, erythematous, slightly keratotic plaques covering the dorsal skin of the finger joints in the son.

DISCUSSION

We have described a mother and son with the Huriez syndrome. Mother developed three squamous cell carcinomas in the affected dorsal skin of the left hand.

Until now seven families and one solitary case have been described, including the original description by Huriez (1-9). Malignant degeneration of the affected skin was observed in 11/86 affected family members (average 13%). Four of them had died from metastasis of their cutaneous malignancy. This unusually high mortality rate of 5% might be explained by the frequently low grade of differentiation. As several affected family members described were often young, the lifetime risk is probably even higher. Hamm et al. found a virtual absence of epidermal Langerhans cells in the involved skin only. The resulting diminution of recognition and presentation of tumour-associated antigens, might contribute to the proneness to malignant degeneration (8).

Yesudian et al. described a family with palmoplantar keratoderma clinically identical to that observed in the Huriez syndrome. In 3/22 tylotic family members squamous cell carcinomas were found in the affected skin, and one of these died of squamous cell carcinoma of the oesophagus (10). A similar association with internal malignancies has not been described in other patients. Malignant degeneration of the tylotic skin has also been observed in the Schöpf-Schulz-Passarge syndrome, which can be differentiated easily from the Huriez syndrome (11). No other palmoplantar keratodermas are known to be associated with the development of squamous cell carcinomas of the affected skin.

The enhanced risk of development of squamous cell carcinoma on the affected skin at young age, in the syndrome of Huriez, underlines the statement that the accurate specification and diagnosis of an individual patient presenting with a hereditary palmoplantar keratoderma is not an academical exercise but important for individual patient management to offer optimal treatment and follow-up in those at risk of skin cancer. As we described recently, specific morphology and distribution of the hyperkeratosis, the inheritance pattern and the presence or absence of associated features, are the most important features enabling a rapid classication. Additional criteria are the presence of skin lesions on areas other than the palms and soles, the age of onset of the keratoderma, the severity of the disease process and the histological findings (12). Concerning treatment of the Huriez syndrome, almost no data are available. Retinoids have been used succesfully in several other palmoplantar keratodermas (13-17). Furthermore, there is ongoing evidence that retinoids are effective as profylaxis against the development of neoplasias of the skin (18,19). Until now, only one patient with the Huriez syndrome has been treated with retinoids for 5 years. This patient did not develop any further squamous cell carcinomata in this period (20).

In conclusion, patients with the Huriez syndrome should be regarded at risk for the development of squamous cell carcinomas on the affected skin. Therefore, regular follow-up and explicite patient instruction are a prerequisite. Treatment with retinoids might be considered.

REFERENCES

1. Huriez CL, Agache P, Bombart M, et al. Epitheliomas spinocellulaires sur atrophie cutanée congénitale dans deux familles à morbidité cancéreuse élevée. Bull Soc Fr Derm Syph 1963;70:24-28.
2. Huriez CL, Agache P, Souillart F, et al. Scléroatrophie familiale des extrémités avec dégénérescence cellulaires multiples. Bull Soc Fr Derm Syph 1963;70:743-4.
3. Huriez CL, Deminati M, Agache P, et al. Génodermatose scléro-atrophiante et kératodermique des extrémités. Ann Dermatol Syphiligr 1969;96:135-46.
4. Lambert D, Planche H, Chapuis JL. La génodermatose scléro-atrophiante et kératodermique des extrémités. Ann Dermatol Vénéréol 1977;104:654-7.
5. Fischer S. La génodermatose scléro-atrophianta et kératodermique des extrémités. Ann Dermatol Venereol 1978;105:1079-1082.
6. Shaw M, Formentini E, de Kaminski AR. Genodermatosis escleroatrofiante y queratodermica de las extremidades frecuentemente degenerativa. Med Cut Ibero Lat Am 1978;5:291-296.
7. Kavanagh GM, de Berker D, Jardine P, Peachey RD. The scleroatrophic syndrome of Huriez. Br J Dermatol 1993;129 (Suppl.42):21 (Abstr.).
8. Hamm H, Traupe H, Bröcker E, Schubert H, Kolde G. The scleroatrophic syndrome of Huriez: a cancer-prone genodermatosis. Br J Dermatol 1996;134:512-518.
9. Patrizi A, Lernia VD, Patrone P. Palmoplantar keratoderma with sclerodactyly (Huriez syndrome). J Am Acad Dermatol 1992;26:855-7.

10. Yesudian P, Premalatha S, Thambiah AS. Genetic tylosis with malignancy: a study of a South Indian pedigree. Br J Dermatol 1980;102:597-600
11. Küster W, Hammerstein W. Das Schöpf-Syndrom. Klinische, genetische und lipidbiochemische Untersuchungen. Hautarzt 1992;43:763-766.
12. Lucker GPH, van de Kerkhof PCM, Steijlen PM. The hereditary palmoplantar keratoses: an updated review and classification. Br J Dermatol 1994;131:1-14.
13. Blanchet-Bardon C, Nazarro V, Rognin C, Geiger JM, Puissant A. Acitretin in the treatment of severe disorders of keratinization. Results of an open study. J Am Acad Dermatol 1991;24:982-986.
14. van de Kerkhof PCM, van Dooren-Greebe RJ, Steijlen PM. On the efficacy of acitretin in keratodermia palmaris et plantaris transgrediens et progrediens Greither. Eur J Dermatol 1992;2:503-505.
15. Rivers JK, Duke EE, Justus DW. Etretinate: management of keratoderma hereditaria mutilans in four family members. J Am Acad Dermatol 1985;13:43-49.
16. Dupré A, Christol B, Bonafé JL, Touron P. Pachyonychie congénitale. Description de 3 cas familiaux. Traitement par le rétinoïde aromatique (RO 10.9359). Ann Dermatol Venereol 1981;108:145-149.
17. Haußer I, Frantzmann Y, Anton-Lamprecht I, Estes S, Frosch PJ. Olmsted Syndrom. Erfolgreiche Therapie durch Behandlung mit Etretinat. Hautarzt 1993;44:394-400.
18. Lever L, Marks R. Topical retinoid treatment for skin cancer: a review. Skin Pharmacol 1991;4:125-131.
19. Costa A, Formell F, Chiesa F, Decensi A, de Palo G, Veronesi U. Prospects of chemoprevention of human cancers with the synthetic retinoid fenretinide. Cancer Res 1994;54:2032s-2037s.
20. Delaporte E, Guyen-Mailfer CN, Janin A, Savary JB, Vasseur F, Feingold N, Piette F, Beroend H. Keratoderma with scleroatrophy of the extremities or sclerotylosis (Huriez syndrome): a reappraisal. Br J Dermatol 1995;133:409-416.

Chapter 6

Topical calcipotriol in the treatment of epidermolytic palmoplantar keratoderma of Vörner.

GPH Lucker
PCM van de Kerkhof
PM Steijlen

Br J Dermatol 1994;130:543-545.

INTRODUCTION

Hereditary epidermolytic palmoplantar keratoderma (HEPPK) is a genodermatosis with an autosomal dominant mode of inheritance, which was first described by Vörner in 1901 [1]. The clinical features and mode of transmission are similar to those of palmoplantar keratoderma (PPK) diffusa circumscripta of Unna-Thost. The dermatosis usually appears in the first weeks of life as a palmoplantar erythema which subsequently becomes covered by thick hyperkeratosis. The hyperkeratosis starts at the margins of the palms and soles, extends towards the centre, and finally covers the whole surface with a uniformly thick horny layer. In addition, hyperkeratotic lesions may develop on the dorsa of the joints of the fingers and toes [2], and on the dorsa of the hands [3], but the borders of the affected areas on the palms and soles are sharply demarcated, and there is no transgression to the dorsal surfaces. In the early stages of the disorder, a violaceous border is present, but this usually resolves after several years. Hyperhidrosis may be present, but is not a constant feature. Blisters may occur on the affected areas, and may suggest the diagnosis [2,4-6]. Histological examination reveals the features of epidermolytic hyperkeratosis, and distinguishes HEPPK of Vörner from PPK diffusa circumscripta of Unna-Thost.

Treatment with aromatic retinoids reduces the hyperkeratosis and increases the tactile sensitivity [2], but pronounced sensitivity and vulnerability of the skin on the palmoplantar surfaces, resulting in discomfort and even erosions, may necessitate discontinuation of the treatment. We report the clinical efficacy of topical calcipotriol treatment in a patient with HEPPK of Vörner.

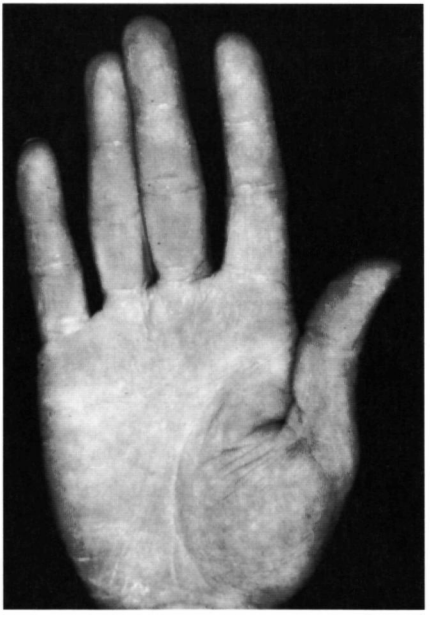


Fig. 1 Before therapy an evenly thick, yellowish hyperkeratosis is visible covering diffusely the palmar skin.

CASE REPORT

A 38-year-old man presented with hyperkeratosis of the palms and soles, which had been present since he was 4 weeks old. On examination, there was diffuse, thick, yellowish hyperkeratosis confined to the palms and soles, with a shrply defined margin (Fig. 1). Erythema was not evident at the borders of the affected areas. The dorsa of the hands and feet were not involved. There were no areas of hyperkeratosis elsewhere on the body, and his hair, teeth, and mucous membranes were normal. He complained of significant hyperhidrosis, which had diminished with increasing age. He found that walking barefoot was uncomfortable, as was heavy manual work. Blisters had never been present.

His family history revealed that nine individuals in four consecutive generations had suffered from the same dermatosis, which is consistent with an autosomal dominant mode of inheritance.

Histology of a biopsy specimen from the palmar skin revealed orthokeratotic hyperkeratosi, focal parakeratosis, a prominent granular cell layer, and acanthosis. In the thickened stratum granulosum, keratohyalin granules appeared clumped, the cells displayed numerous vacuoles, and cell walls were ruptured (Fig. 2). These findings are consistent with epidermolytic hyperkeratosis.

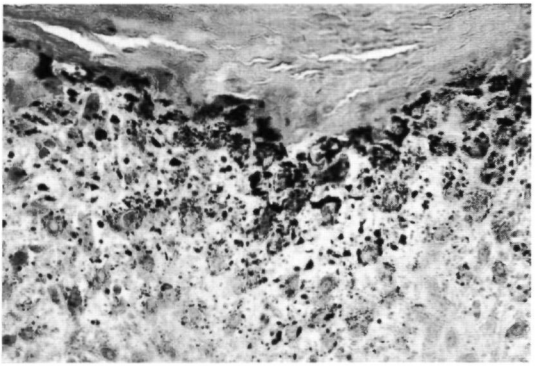


Fig. 2 Clumpy keratohyalin granules, numerous vacuoles and ruptured cell walls in the granular cell layer. (hematoxylin-eosin, x400)

Using a bilaterally paired comparative approach, calcipotriol 50 μg/g and urea 40mg/g, both in an ointment base, were applied twice daily to the right and left hands, respectively. Within a month of starting treatment there was focal reduction of hyperkeratosis on the right hand (Fig. 3). Improvement of tactile sense was marked. As a result of increased tactile sensitivity, the application frequency was reduced to once daily 3 times a week, after 4 weeks of treatment. Clinical evaluation at 3 months revealed a remarkable unilateral improvement in favour of the calcipotriol-treated side (Fig. 4 a and b).

Despite the fact that the increased sensitivity could be maintained at an acceptable level by reduction of the application frequency, the patient discontinued treatment after 4 months, because he considered the pretreatment state was preferable to the marked sensitivity of the skin which accompanied a reduction in the degree of hyperkeratosis.

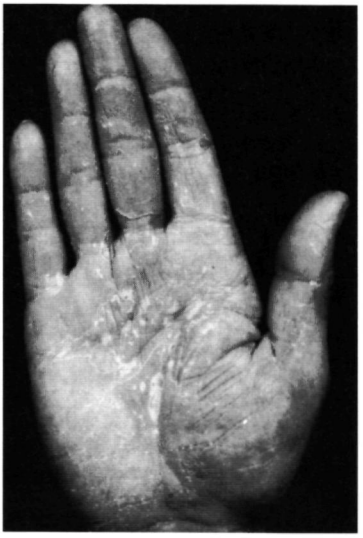
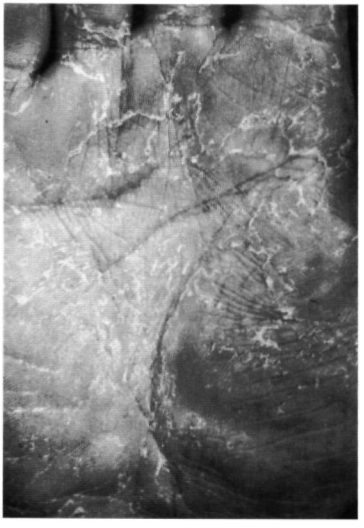


Fig. 3 Intermediate stage, 4 weeks after onset of treatment with calcipotriol. A patchy red reduction of hyperkeratosis is seen at the calcipotriol treated hand.



a b

Fig. 4 Clinical results after 3 months of treatment. **a.** Hyperkeratosis has almost completely disappeared at the calcipotriol treated right hand. **b.** The left hand still shows a diffuse hyperkeratosis comparatible with the pre-treatment observations.

DISCUSSION

As far as we are aware, this case represents the first report of a beneficial response of HEPPK of Vörner to treatment with topical calcipotriol.

Until recently PPK diffusa circumscripta Unna-Thost was believed to be the most

frequent type of hereditary PPK, whereas HEPPK of Vörner was considered to be a rare disorder. Hamm et al. [7], reviewed a group of 21 patients with diffuse PPK and found the features of epidermolytic hyperkeratosis in 12 of them, confirming the diagnosis of HEPPK. None of the patients was classified as PPK of Unna-Thost. Reinvestigation of the family originally seen by Thost also revealed the features of epidermolytic hyperkeratosis [8]. The true frequency of HEPPK is therefore probably underestimated, and possibly this dermatosis may represent the most frequent type of hereditary PPK.

Treatment options for HEPPK are limited. Systemic retinoids produce disabling tenderness of the skin, and topical treatments are of modest value. Our case demonstrates that calcipotriol (50 μg/g) ointment may be effective in the treatment of HEPPK. The increased sensitivity of palms and soles which accompanies the reduction in hyperkeratosis can be controlled by adjusting the application frequency, and topical calcipotriol may therefore be a promising approach to the treatment of HEPPK of Vörner.

REFERENCES

1. Vörner H. Zur Kenntnis des Keratoma hereditarium palmare et plantare. Arch Dermatol Syph (Berlin) 1901;56:3-31.
2. Fritsch P, Hönigsmann H, Jaschke E. Epidermolytic hereditary palmoplantar keratoderma. Report of a family and treatment with an oral aromatic retinoid. Br J Dermatol 1978;99:561-568.
3. Kanitakis J, Tsoitis G, Kanitakis C. Hereditary epidermolytic palmoplantar keratoderma (Vörner). Report of a familial case and review of the literature. J Am Acad Dermatol 1987;17:414-422.
4. Goette DK. Familial congenital epidermolytic hyperkeratosis confined to the palms and soles. South Med J 1974;67:1126-1128.
5. Berth-Jones J, Hutchinson PE. A family with palmoplantar epidermolytic hyperkeratosis. Clin Exp Dermatol 1989;14:313-316.
6. Requena L, Schoendorff, Sanchez Yus E. Hereditary epidermolytic palmo-plantar keratoderma (Vörner type)-report of a family and review of the literature. Clin Exp Dermatol 1991;16:383-388.
7. Hamm H, Happle R, Buterfass T, Traupe H. Epidermolytic Palmoplantar Keratoderma of Vörner: Is It the Most Frequent Type of Hereditary Palmoplantar Keratoderma? Dermatologica 1988;177:138-145.
8. Küster W, Becker A. Indication for the identity of Palmoplantar Keratoderma Type Unna-Thost with Type Vörner. Thost's family revisited 110 years later. Acta Derm Venereol (Stockh) 1992;72:120-122.

Chapter 7

The hereditary palmoplantar keratoses: an updated review and classification.

GPH Lucker
PCM van de Kerkhof
PM Steijlen

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SUMMARY

The palmoplantar keratoses (PPKs) comprise a heterogeneous group of disorders of keratinization, which can be subdivided into hereditary and acquired forms. Many authors have attempted to classify the hereditary forms [1-5], and most classifications have been based on the morphology, distribution, associated symptoms and mode of inheritance. Subsequently, many new forms have been recognized, and what were previously considered to be distinct types have been shown to be variants of a single type, both of which limit the usefulness of previous classifications. Hence, we propose a new, updated classification, which enables accurate diagnosis of these disorders.

INTRODUCTION

The disorders known as palmoplantar keratoses (PPKs) comprise a number of different clinical entities, and there are both hereditary and acquired forms. Hereditary forms may be localized primarily to the hands and feet or be associated with more generalized skin diseases, such as autosomal dominant ichthyosis vulgaris, bullous ichthyosiform erythroderma, lamellar ichthyosis and erythrokeratoderma. Classification of the hereditary PPKs localized primarily to the hands and feet is difficult, because of inter- and intraindividual variations, differences in nomenclature, and the large number of reported cases. In recent years, a number of new forms have been described, and forms which were previously considered to be distinct have been shown to be variants of the same type of keratoderma. Based on the available data, we propose an updated classification (Table I). The most important features in the classification of PPKs are the specific morphology and distribution of the hyperkeratosis, the presence or absence of associated features, and the inheritance pattern. Additional criteria are the presence of skin lesions on areas other than the palms and soles, the age at onset of the keratoderma, the severity of the disease process, and the histopathological findings.

DIFFUSE HEREDITARY PPKs WITHOUT ASSOCIATED FEATURES

PPK diffusa circumscripta Unna-Thost

Diffuse PPK Unna-Thost [6,7] usually presents in the first month of life, and is evident by the age of 2 in the majority of cases. An even, very thick, diffuse hyperkeratosis covers the palms and soles, starting at the margins and extending to the centre. Initially, the margins show a violaceous border, which usually disappears after several years. There is no spread on to the extensor surfaces. Aberrant keratotic lesions may appear on the dorsa on the hands and feet, and the knees and elbows. Marked hyperhidrosis is usual. The nails may be thickened. Histologically, PPK Unna-Thost is said to show non-specific changes, but there are no case reports in the literature confirming such histological findings. Until recently, diffuse PPK Unna-Thost was generally believed to be the most frequent type of hereditary PPK. Clinically, the disease is identical to PPK Vörner, which is characterized histologically by epidermolytic hyperkeratosis. In a number of cases clinically thought to have PPK Unna-Thost, the histological features were those of epidermolytic hyperkeratosis [8]. Reinvestigation of the family originally seen by Thost also revealed the histological features of epidermolytic hyperkeratosis [9]. Hence, there is doubt about the existence of PPK Unna-Thost as a separate entity.

Table I. Hereditary palmoplantar keratoses

	Inheritance	No associated features	Associated features
Diffuse	AD	Unna-Thost Greither Vörner Sybert	Vohwinkel Howel-Evans Huriez Clouston PPK and sensorineural deafness Olmsted
	AR	Mal de Meleda Gamborg Nielsen Nagashima Acral keratoderma	Papillon-Lefèvre Bureau-Barrière-Thomas
Nummular/Linear	AD	Wachters PPK nummularis	Richner-Hanhart Pachyonychia congenita Focal palmoplantar and oral mucosa hyperkeratosis syndrome
	AR		Pachyonychia congenita Jakac-Wolf
Papular	AD	Davies-Colley Acrokeratoelastoidosis Focal acral hyperkeratosis	Hanhart
	AR		Schöpf-Schulz-Passarge

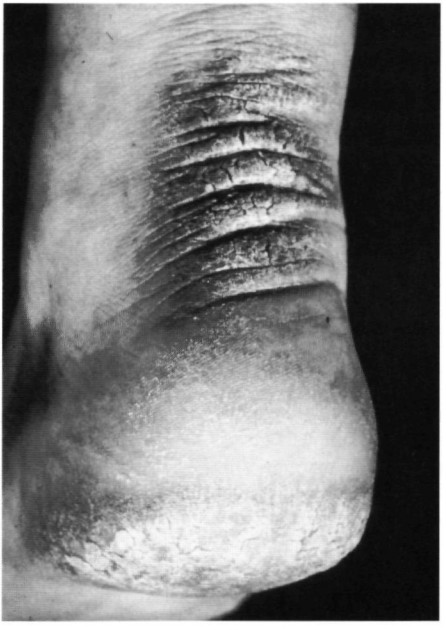

Fig. 1 PPK transgrediens et progrediens Greither, showing diffuse hyperkeratosis covering the heel and the region of the Achilles tendon. Spreading to the dorsal surfaces is similar to mal de Meleda, shown in figure 4 and 5.

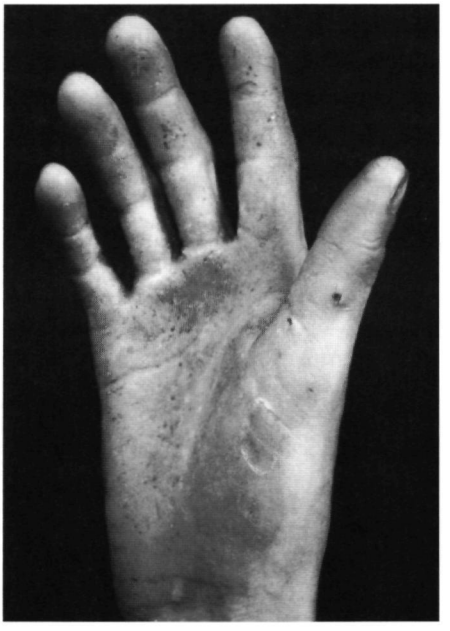

Fig. 2 PPK cum degeneratione granulosa Vörner, exhibiting thick hyperkeratosis covering diffusely the palms. The characteristic histopathological picture is shown in figure 3.

PPK transgrediens et progrediens Greither (Fig. 1)

PPK Greither is a diffuse PPK which extends on to the dorsa of the hands and feet (transgrediens), has a violaceous border, and is associated with hyperhidrosis [10-14]. Additional hyperkeratotic lesions can be seen on the knees and elbows, and in the region of the Achilles tendon. Worsening of the keratosis is seen during childhood, followed by a static phase after puberty, and finally a tendency to improve in the fifth decade. It differs from the Unna-Thost variety by extending to the dorsal surfaces, and from mal de Meleda by its dominant inheritance and variable intrafamilial expression. Solitary cases must be differentiated from erythrokeratodermas.

PPK cum degeneratione granulosa Vörner (Figs 2, 3 and 4)

The clinical features and the mode of transmission of PPK Vörner [15] are the same as those of PPK Unna-Thost. It differs from the latter in that epidermolytic hyperkeratosis is a histological feature of PPK Vörner. To date, at least 33 families with this disorder and 11 sporadic cases have been reported [16]. Epidermolytic hyperkeratosis also occurs in several other skin conditions, such as bullous congenital ichthyosiform erythroderma Brocq, ichthyosis bullosa Siemens, and some linear epidermal naevi. PPK nummularis is another PPK characterized by epidermolytic hyperkeratosis [17]. In this disorder, the involvement is not diffuse, but mainly confined to the pressure points. Within the group of diffuse hereditary PPKs, however, PPK Vörner is so far considered to be the only type showing this distinctive histological feature. Consequently, a correct classification of diffuse PPK is impossible without histological examination. PPK Vörner appears to be the most frequent type of hereditary PPK [8].

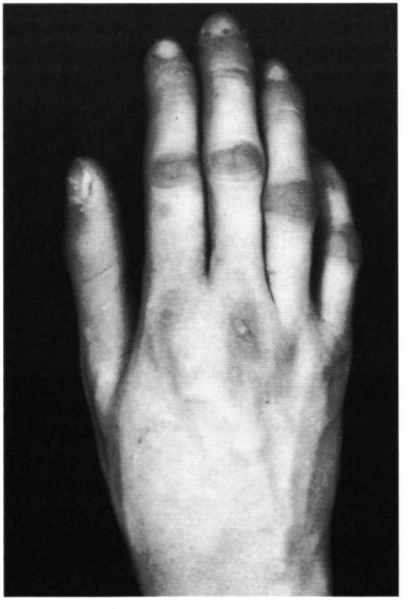

Fig. 3 Same patient as in figure 2. Additional hyperkeratotic plaques are present at the dorsal surfaces of the proximal interphalangeal joints.

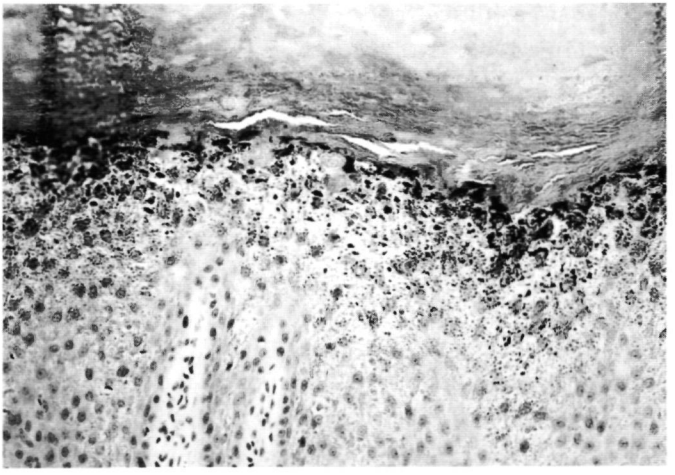

Fig. 4 Hyperkeratosis, hypergranulosis and acanthosis. The granular cell layer displays numerous clumpy keratohyalin granules, vacuoles and ruptured cell walls, consistent with epidermolytic hyperkeratosis (hematoxylin-eosin, x400).

PPK of Sybert

In 1988, Sybert described a family with a palmoplantar keratoderma which started with erythema and scaling of the palms and soles in the first year of life [18]. The palmoplantar lesions eventually progressed to a severe diffuse hyperkeratosis, causing deformities and spontaneous amputation of the digits. In childhood, additional lesions developed in the natal cleft and groins. With increasing age, hyperkeratoses developed on the elbows, knees, dorsa of the hands and feet, posterior aspects of the forearms and anterior aspects of the legs. The pedigree was consistent with an autosomal dominant mode of inheritance. This diffuse, transgrediens, mutilating PPK has to be differentiated from mal de Meleda, PPK of Gamborg Nielsen and PPK of Greither. The clinical features resemble those found in mal de Meleda which, however, is an autosomal recessive disorder. PPK of Gamborg Nielsen shows less extension on to dorsal surfaces, fewer additional hyperkeratoses, and is recessively inherited. The severity and extent of involvement are much greater than in PPK of Greither.

Mal de Meleda (Keratosis extremitatum hereditaria transgrediens et progrediens) (Figs 5 and 6)

This very rare disorder has, as a result of inbreeding, an endemic prevalence on the adriatic island of Mljet [19-21]. It is characterized by a diffuse, thick hyperkeratosis with a prominent erythematous border. The thick hyperkeratosis may lead to flexion contractures. The disease has its onset in early infancy, and follows a progressive course, with extention on to the dorsal surfaces of hands and feet. Constricting bands surrounding the digits are typical [22], and occasionally result in spontaneous amputation

[23]. Concomitant lesions can be found on other sites, especially the elbows and knees [24]. Perioral erythema and hyperkeratosis may be present [25], resembling the clinical features of Olmsted syndrome. Hyperhidrosis of the affected parts, with maceration of the hyperkeratotic masses and consequent production of a rancid odour, is pronounced. Nailchanges (koilonychia, nail thickening, subungual hyperkeratosis) are usually present. The disease has to be differentiated from PPK transgrediens et progrediens Greither and PPK of Sybert, both of which have a dominant mode of inheritance, and from PPK of Gamborg Nielsen.

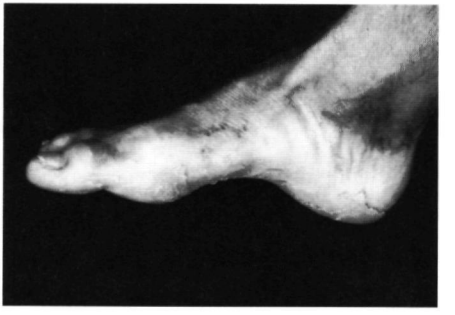

Fig. 5 Mal de Meleda displaying a diffuse hyperkeratosis with a prominent erythematous rim, spreading to the dorsal surfaces as shown in figure 6.

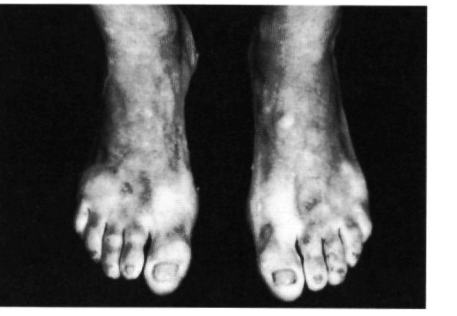

Fig. 6 Same patient as in figure 5. The dorsal surfaces are covered by hyperkeratosis, extending from the plantar surfaces. The hyperkeratosis is surrounded by a border of erythema.

PPK Gamborg Nielsen

Gamborg Nielsen delineated a severe form of PPK, represented by six patients in two families, who lived in the northernmost county of Sweden [26]. This type of PPK was characterized by a very thick hyperkeratosis, distinctly demarcated from normal skin. The dorsal aspects of the finger joints were covered by hyperkeratotic plaques. A violaceous margin was present in four patients. One patient demonstrated extension of the keratoderma on to the dorsa of the hands. Another featured mutilating changes due to constricting bands surrounding the fingers [27]. Apart from knuckle pads on the dorsa of the fingers, there were no additional hyperkeratoses. The pedigree in both families was consistent with an autosomal recessive mode of inheritance. The disease has to be differentiated from PPK of Sybert, PPK of Greither, and from mal de Meleda. PPK Sybert exhibits more extension on to dorsal surfaces, and distant keratoses, and is

inherited as an autosomal dominant trait. Compared with mal de Meleda, the Gamborg Nielsen type displays less severe hyperkeratoses, no nail deformities, and no distant keratoses, except for the knuckle pads. Similar, although less severe, cases have been described in the Japanese literature as the "Nagashima type" [28].

Acral keratoderma

Three siblings have been reported who displayed a keratoderma characterized by diffuse and striate hyperkeratosis of the palms and soles, hyperkeratotic plaques on the dorsa of the hands and toes, and linear hyperkeratotic lesions over the Achilles tendons, ankles, elbows and knees. In one individual, the right fifth toe was missing, and a constricting band completely encircled the base of the left fifth toe. Histology showed dyskeratotic changes without epidermolytic hyperkeratosis. Clinically, the keratoderma can be distinguished from PPK mutilans by the presence of striate hyperkeratosis on the palmar surface of the fingers, the absence of starfish-shaped hyperkeratosis, and the lack of a violaceous border surrounding the palmoplantar keratoderma. In addition, the pedigree suggests an autosomal recessive inheritance pattern. The typical clinical features enable a distinction to be made between acral keratoderma and mal de Meleda [29].

DIFFUSE HEREDITARY PPK'S WITH ASSOCIATED FEATURES

PPK mutilans Vohwinkel

PPK Vohwinkel is a diffuse PPK with a honeycombed hyperkeratosis, a violaceous border, and hyperhidrosis [30-37]. It presents in infancy, and is characterized by strangulating fibrous bands leading to progressive constriction and eventual spontaneous amputation of the digits. In addition, patients may have distinctive keratotic lesions on the elbows and knees, as well as the dorsa of the hands and feet, with a peculiar, linear and starfish-shaped configuration. In one patient, grossly mutilating keratoderma was accompanied by keratosis of the groins and perianal skin [38]. A number of associated features have been noted to occur sporadically: alopecia [35,38], high-tone acoustic impairment [35,37], deafness [33], spastic paraplegia, myopathy [37], ichthyosiform dermatoses [31,32,39], and nail anomalies. Constriction bands on digits may occasionally occur in mal de Meleda, pachyonychia congenita, acral keratoderma, the Olmsted syndrome and PPK of Sybert.

Carcinoma of the oesophagus with keratosis palmaris et plantaris (Howel-Evans)

Howel-Evans et al. reported the association of palmoplantar keratoderma with carcinoma of the oesophagus [40]. In their study of two Liverpool families, oesophageal carcinoma occurred in 18 of 48 tylotic family members, but in only one of 87 non-tylotic members. The age of onset of the palmoplantar hyperkeratosis was between 5 and 15 years, and the average age of onset of the oesophageal carcinoma 43 years. Yesudian et al. described a pedigree with tylosis in 22 members of a family spanning over 5 generations [41]. Of

those affected, three of the fourth generation tylotics developed squamous carcinoma of the tylotic skin, and one of these died from carcinoma of the oesophagus. Palmoplantar keratosis was present at birth. Skin cancer developed in the second decade of life. It is possible that there is a genetic association between the state of the oesophagus and the palmoplantar keratoderma which predisposes both sites to the development of squamous carcinoma. Acquired PPK, beginning late in life and apparently not genetically determined, has also been described in association with internal malignancy [42-46].

PPK with sclerodactyly (Huriez syndrome)

The disorder described first by Huriez et al [47-49] is characterized by sclerodactyly, diffuse keratoderma of the palms and soles, nail anomalies (aplasia, ridging and clubbing) and possible malignant degeneration of the affected skin. Associated hypohidrosis is common. Another two familial cases have been reported [50,51]. The disease is present at birth, or appears in the first years of life, and persists unchanged throughout adult life. Sclerodactyly, not associated with Raynaud's phenomenon, is the most prominent feature of the syndrome. In some severe PPKs there is a suggestion of sclerosis, but in the Huriez syndrome the sclerotic changes are disproportionately greater. Squamous cell carcinomas often develop in the atrophic skin, as early as the third to fourth decades of life. The Huriez syndrome can be distinguished from scleroderma by its onset at birth, absence of systemic signs and symptoms, lack of vasomotor phenomena, and lack of progression during adulthood.

Hidrotic ectodermal dysplasia (Clouston syndrome)

Hidrotic ectodermal dysplasia, first described by Clouston in 1928, is characterized by dystrophy of the nails, defects of the hair, and palmoplantar keratosis [52-55]. There is sparsity of the hair of the scalp, face, eyebrows, eyelashes, axillae and genitalia, varies in severity from mild thinning to complete baldness [56]. In some cases, alopecia appears maximally soon after birth, although in the majority hair loss is gradual, less severe, and only occurs after puberty. Hyperkeratosis of the palms and soles has a papillomatous appearance, with multiple small fissures, and generally increases in severity with age. Skin thickening has also been reported on the knuckles, knees and elbows [56]. Biochemically, a depletion of hair matrix protein, related to a disruption of disulphide bond formation in the keratin of the integumentary system, may account for the clinical features in hair and skin [57]. Sensorineural deafness [58,59], polydactyly, syndactyly, finger clubbing, mental retardation, dwarfism [60], photophobia [57], and strabismus [61], may be associated.

PPK and sensorineural deafness

Recently, Sharland et al. [62] described a new syndrome of diffuse palmoplantar hyperkeratosis invariably associated with a slowly progressive, bilateral, high-frequency,

cochlear hearing loss. The onset of deafness in infancy and early childhood precedes the skin changes, and thereafter both progress slowly with age. The two abnormalities never appeared as isolated defects in the family described. A combination of sensorineural hearing loss and palmoplantar keratosis has been recognized previously [63,64]. A variable relationship between the inheritance of skin lesions and acoustic impairment has been reported in the Olmsted syndrome [65], and PPK mutilans Vohwinkel [35]. The Clouston syndrome also includes other ectodermal defects [52].

Mutilating palmoplantar keratoderma with periorificial keratotic plaques (Olmsted syndrome)

Including the original description by Olmsted [65], there are five well documented cases of this syndrome [65-68]. The syndrome consists of congenital, diffuse, sharply marginated keratoderma of the palms and soles, with flexion deformities of the digits, leading to constriction or spontaneous amputation, periorificial keratoses, and onychodystrophy. Perianal involvement has been reported in four cases [38, 65-67]. Leukokeratosis was present in two cases [66,67], and in one patient the groins and inner thighs, ears and anterior neck, were all involved by the age of 2 years. This patient also had universal alopecia, absence of a premolar tooth, joint hypermobility and, at the age of 20, linear hyperkeratotic streaks in the anticubital fossae and on the flexor aspects of the forearms [67]. Recently, a case has been reported with a congenital non-mutilating PPK and nail dystrophy, who developed progressive perioral and perineal keratoderma and, in addition, bilateral corneal epithelial dysplasia, leading to severe corneal scarring and impairment of vision [69]. The principal genetic syndromes to be excluded in the differential diagnosis include hidrotic ectodermal dysplasia of the Clouston type, pachyonychia congenita, mal de Meleda, and PPK of Vohwinkel. The condition mimics acrodermatitis enteropathica, which can be excluded by determination of the plasma zinc level.

Palmoplantar keratoderma with periodontitis (Papillon-Lefèvre syndrome)

The Papillon-Lefèvre syndrome (PLS) is characterized by a diffuse transgrediens palmoplantar keratosis and premature loss of both the deciduous and permanent teeth [70]. In addition to the palmoplantar hyperkeratosis, many PLS patients have scaly erythematous lesions over the knees, elbows, and interphalangeal joints, not uncommonly misdiagnosed as psoriasis [71]. Redness and thickening of the palms and soles usually appear in the first years of life, at the time of eruption of the deciduous teeth. A spontaneous improvement parallels the resolution of gingival inflammation after the loss of the permanent teeth. Associated hyperhidrosis causes an unpleasant odour [72]. An increased susceptibility to infections has been observed in about 20% of PLS patients [73]. The skin is reported to be the most common site affected by infections; internal organs are less frequently involved [74]. Disorders of leucocyte function might account

for the prominent gingival and cutaneous infections. Some investigators have demonstrated disturbances in both polymorphonuclear leucocyte motility and bactericidal functions [75-77] or in bactericidal functions alone [78-80], whereas others have not found any defects in leucocyte function [81,82]. The precise underlying mechanism responsible for susceptibility to infections in PLS patients, however, remains to be determined. Histopathological changes are non-specific. Electron microscopic features include lipid-like vacuoles in the corneocytes and granulocytes, a reduction in tonofilaments, and irregular keratohyalin granules. A syndrome combining the features of PLS with flat feet, onychogryphosis, arachnodactyly and acro-osteolysis, has been described in one family by several authors [83-85]. PLS has to be distinguished from the Schöpf-Schulz-Passarge syndrome.

PPK with clubbing of the fingers and toes and skeletal deformity (Bureau, Barrière, Thomas)

In 1959, Bureau, Barrière and Thomas described four members of one family who presented with a diffuse, symmetrical, non-transgrediens palmoplantar keratosis, clubbing of the fingers and toes, and skeletal changes consisting of bone hypertrophy and thinning of the cortex of long bones [86,87]. Hedstrand et al. reported two sisters with consanguineous parents, who developed palmoplantar keratoderma in childhood, clubbing of fingers and toes, and unusual skeletal changes in the terminal phalanges. X-rays showed a peculiar deformity of the terminal phalanges. The distal ends appeared splayed, and showed marginal effects suggesting atrophy [88]. In both of these families the PPK was accompanied by marked hyperhidrosis. Recently, a patient was described with PPK, drumstick-fingers, hypotrichosis, hypohidrosis and dental dysplasia [89].

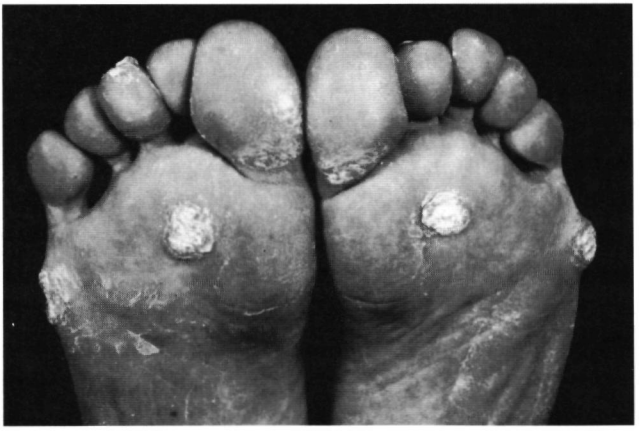

Fig. 7 PPK varians of Wachters, displaying characteristic nummular hyperkeratotic plaques, mainly confined to the pressure points.

NUMMULAR HEREDITARY PPKs WITHOUT ASSOCIATED FEATURES

Keratosis palmoplantaris varians Wachters (Fig. 7)

Originally, distinct subtypes were described by Fuhs [90], Brünauer [91], and Siemens [92], because of the diversity of clinical features. All of these variants, however, should be considered as one dermatosis, for which Wachters introduced the term 'keratosis palmoplantaris varians' [93]. A characteristic feature is the great inter- and intra-familial variability. The palmar keratoses have either a nummular, linear, membranaceous, fissured or periungual configuration. The keratoses on the soles always have a nummular appearance, and are localized on the pressure points [92,93]. Woolly hair has been described as an associated feature in one family [94]. Nevertheless, because of the absence of associated features in other families [90-93,95,96] PPK varians Wachters is classified in the group of nummular-linear PPKs without associated features.

Keratosis palmoplantaris nummularis ('hereditary painful callosities') (Fig. 8)

Nummular keratotic lesions, almost exclusively located on the plantar pressure points, with pain as the major complaint, are characteristic of this disease, and have been reported in 34 patients in 14 families [97-103]. The lesions usually appear when an affected child begins to walk. They progress slowly, and are often accompanied by pain. The palms may also be involved after mechanical trauma. Lesions in areas other than the palms and soles have been observed in only two patients [101,102]. The major histological feature, in nearly all patients, is local epidermolytic hyperkeratosis [102]. This disorder differs from PPK Vörner in that in the Vörner type of palmoplantar keratosis there is diffuse palmoplantar epidermolytic hyperkeratosis.

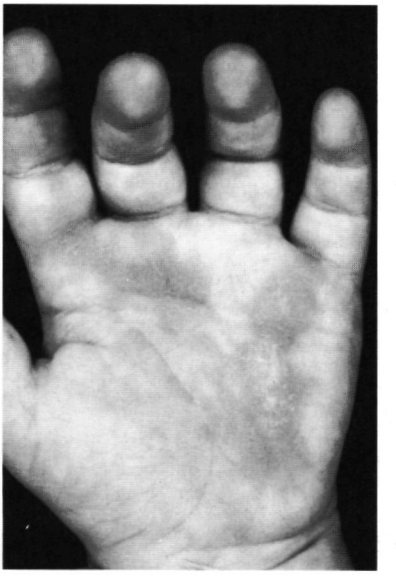

Fig. 8
PPK nummularis. Confluent nummular hyperkeratosis displaying epidermolytic hyperkeratosis as shown in figure 3 in a focal pattern.

NUMMULAR HEREDITARY PPKs WITH ASSOCIATED FEATURES

Tyrosinaemia type II (Richner-Hanhart syndrome)

Tyrosinaemia type II is a rare disorder of tyrosine metabolism, characterized clinically by focal, painful, palmoplantar keratoses, bilateral pseudoherpetic corneal ulceration and mental retardation [104-107]. The typical cutaneous changes of tyrosinaemia type II consist of painful, circumscribed, hyperkeratotic plaques on the palms and soles. Occasionally, aberrant hyperkeratotic lesions are present in areas such as the elbows and knees, or even the tongue [108]. Hyperhidrosis of the palms and soles is frequently associated [109-111]. Mild herpetiform corneal erosions and dendritic ulcers develop within the first months of life, and may lead to corneal scarring and glaucoma [112]. Skin lesions usually occur when eye lesions have developed, although the skin lesions may be present without eye lesions [113]. Deficiency of the enzyme tyrosine aminotransferase, which leads to increased serum levels of tyrosine and phenolic acid metabolites of tyrosine, is the biochemical basis for tyrosinaemia type II [108,114,115]. Histologically, eosinophilic cytoplasmic inclusions are present in the Malpighian layer of a thickened epidermis [109].

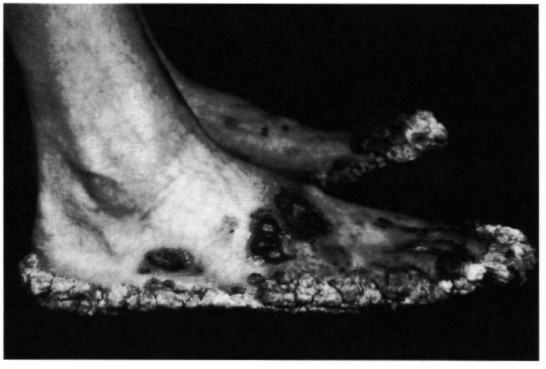

Fig. 9
Pachyonychia congenita, exhibiting severe naildystrophy, a diffuse warty palmoplantar keratosis and nummular keratoses on the dorsal surfaces.

Pachyonychia congenita (Fig. 9)

Pachyonychia congenita was originally described by Muller in 1904 [116] and Wilson in 1905 [117], although the association with palmoplantar keratoderma and other ectodermal defects was first reported by Jadassohn and Lewandowski [118]. It is characterized by discoloration and thickening of the nails, usually beginning within the first month of life. The thickening is the result of subungual hyperkeratosis, with an upward angulation of the distal part of the nail plate, whereas the lateral borders are often incurved. Dystrophy of all the nails, and dyskeratotic skin lesions are necessary to establish the diagnosis. Several subdivisions have been proposed [119-121]. A retrospective study of pachyonychia congenita, performed by Feinstein et al. [122], revealed 168 reported cases. Based on the main features found in this survey, the following classification was

proposed: type I (56% of cases), hyperkeratosis of nails, palmoplantar keratosis, follicular keratosis and oral leucokeratosis; type II (25% of cases), clinical findings of type I, plus bullae of palms and soles, palmar and plantar hyperhidrosis, natal or neonatal teeth, and steatocystoma multiplex; type III (12% of cases), clinical findings of types I and II, plus angular cheilosis, corneal dyskeratosis and cataracts; type IV (7% of cases), clinical findings of types I, II and III, plus laryngeal lesions, hoarseness, mental retardation, hair anomalies and alopecia [122]. However, we recently demonstrated that the presence of other abnormalities is not an absolute prerequisite for the diagnosis [123]. Histopathology of the bullae, which are quite a common manifestation, did not reveal any features of epidermolytic hyperkeratosis [120]. An autosomal recessive form of the disorder has been described [124], and recently late onset pachyonychia congenita has been reported [125].

Focal palmoplantar and oral mucosa hyperkeratosis syndrome

The combination of PPK and hyperkeratosis of the oral mucosa was first described by Fred et al. [126]. Raphael et al. described this combination of clinical features in a family in which there were four affected individuals [127]. There have since been reports of kindreds in which several generations were affected [128-130]. The syndrome is characterized by hyperkeratosis of the palms, soles and oral mucosa. The hyperkeratosis is especially marked on the weight-bearing areas of the soles, areas of the palms exposed to pressure, and the labial attached gingiva. In addition to the attached gingiva, hyperkeratotic lesions develop in areas of the oral mucosa subjected to friction and irritation. The hyperkeratosis, which has a symmetrical distribution, appears in early childhood or around puberty, and the lesions increase in severity with age. However, the severity varies between individuals, and among affected members of the same family. Subungual and circumungual hyperkeratosis may be an associated feature [130]. The syndrome has to be differentiated from others which feature hyperkeratosis of the palms and soles, and oral mucosal lesions, such as PPK Howel-Evans and pachyonychia congenita.

Keratosis palmoplantaris papillomatosa et verrucosa

In 1975, Jakac and Wolf described a clinically distinct keratoderma in four members of one family. This disorder has its onset between 2 and 6 years of age, and is characterized by a verrucous-papillomatous appearance [131]. The PPK, which has a violaceous border, and is nummular at onset, subsequently extends to cover the entire surface of the palms and soles, but does not extend beyond the palmar and plantar surfaces. The skin of the fingers and toes is atrophic, and the digits develop flexion contractures. Aberrant keratotic lesions may be present on the knees, lower arms and buttocks. Profuse hyperhidrosis, which is a constant accompanying feature of the disease, and the pronounced papillomatosis, predispose to secondary infection, and this may lead to

periostitis and osteomyelitis. Although spontaneous remissions have been described, the overall clinical course is progressive. In one of the patients, gingivitis and periodontitis resulted in premature loss of teeth.

PAPULAR HEREDITARY PPKs WITHOUT ASSOCIATED FEATURES

Keratosis palmoplantaris punctata (Davies-Colley, Buschke, Fischer, Brauer) (Figs 10 and 11)

The clinical presentation of numerous tiny keratotic papules, strictly limited to the volar aspects of the hands and feet, has been designated porokeratosis punctata palmaris et plantaris [132,133], palmoplantar keratosis acuminata [134], and punctate porokeratotic keratoderma [135,136], and these numerous apellations have led to much confusion. Lesions usually first develop between the second and fourth decades, with the age of onset ranging from 12 to 70 years. The papular keratoses progress slowly, and remain asymptomatic. Despite great interfamilial clinical variation, there is uniform expression within an affected family. Localized forms limited to the palmar creases have been described [137]. Most of the patients do not have any associated features, but spastic paralysis [138], ankylosing spondylitis [139], and facial sebaceous hyperplasia [134], have been reported in association with PPK punctata. In addition, a coincidental [140], and a possible familial [141], association with gastrointestinal malignancy have been discussed. Histologic examination reveals a compact column of parakeratosis resembling that of a cornoid lamella, but without evidence of dyskeratosis or hydropic degeneration in the epidermis, differentiating the condition from porokeratosis [126,142] a distinction which is important because of the malignant potential of porokeratosis [143].

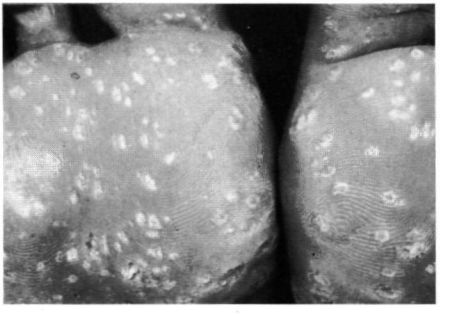

Fig. 10 Classical PPK punctata with multiple keratotic papules covering the soles.

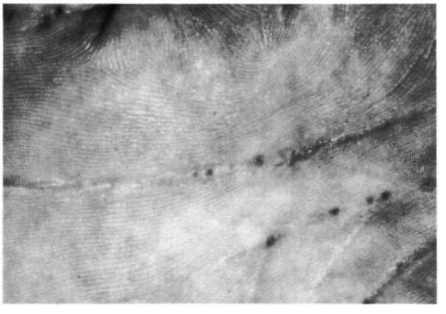

Fig. 11 PPK punctata of the palmar creases. This rare variant of PPK punctata demonstrates small keratotic papules confined to the creases.

Acrokeratoelastoidosis

In 1952, Costa described a clinical entity which he called acrokeratoelastoidosis [144,145]. Clinically, the disease is characterized by small, yellowish, round to oval keratotic papules, mainly confined to the margins of the palms and soles. The keratotic papules may become confluent in the centre of the palms and soles, to produce a diffuse keratoderma. The process begins in adolescence or adult life. The number of papules gradually increases over several years. Local hyperhidrosis is present. Histologically, the disease is characterized by elastorrhexis [144,145]. It must be differentiated histologically from focal acral hyperkeratosis, and clinically from degenerative collagenous plaques of the hands [146], a separate acquired condition, which occurs in an older age group on the sun-exposed parts of the hands.

Focal acral hyperkeratosis (Fig. 12)

This dermatosis is clinically similar to, but histologically different from acrokeratoelastoidosis [147]. It appears to be a focal disorder of keratinization, which has an insidious onset in childhood, reaches a maximum in early life, and causes only cosmetic embarrassment. With the exception of one Arab patient, all reported individuals have been of Negroid racial origin. In addition to the typical papules along the borders of hands and feet, hyperkeratotic papules may be present over the interphalangeal joints of the fingers and toes, and on the heels. On histological examination, there is no elastorrhexis, and this distinguishes the disease from acrokeratoelastoidosis.

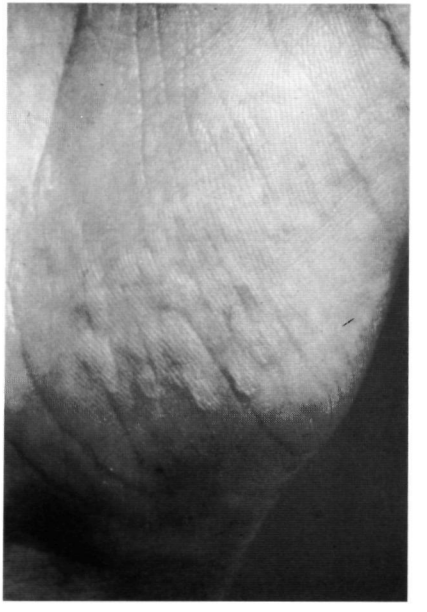

Fig. 12
Focal acral hyperkeratosis displaying a similar picture as acrokeratoelastoidosis, showing yellowish papules mainly confined to the thenar region of the palm.

PAPULAR HEREDITARY PPKs WITH ASSOCIATED FEATURES

Keratosis palmoplantaris with lipomata

In 1947, Hanhart reported a family in which six members in three consecutive generations co-expressed palmoplantar keratosis with lipomata [104]. The keratosis is limited to the palms, soles and volar surfaces of the fingers and toes, and consists of multiple papular keratotic lesions. Its onset is from the third decade of life onwards. The number of lipomata varies from solitary lesions to multiple lipomata in a generalized distribution. However, five members of the same family expressed only palmoplantar keratosis, suggesting a coincidence of two unrelated anomalies in the "affected" members.

Syndrome of cystic eyelids, palmoplantar keratosis, hypodontia and hypotrichosis (Schöpf-Schulz-Passarge syndrome)

Schöpf et al. reported two sisters with a syndrome of cystic eyelids, hypodontia, hypotrichosis and palmoplantar keratosis [148]. A similar grouping of ectodermal defects was reported by Burket et al. in a man who was a sporadic case [149]. Unique in this individual were multiple facial tumours of the follicular infundibulum (Mehregan and Butler) [150]. Happle et al. described the development of squamous cell carcinomata in association with this syndrome [151]. The Papillon-Lefèvre syndrome can be easily distinguished from this disorder. Periodontitis, which is an integral part of the PLS, was not a feature of the reported cases of the Schöpf syndrome, and eyelid cysts have not been described in the PLS.

REFERENCES

1. Franceschetti A, Schnyder UW. Versuch einer klinisch-genetischen Klassifikation der hereditären Palmoplantarkeratosen unter Berücksichtigung assozierter Symptome. Dermatologica 1960;120:154-178.
2. Schnyder UW, Klunker W. Erbliche verhornungsstörungen der Haut. In: Gottron HA, Schnyder UW, eds. Handbuch der Haut- und Geschlechtskrankheiten. Berlin: Springer, 1966: 861-961.
3. Greither A. Erbliche Palmoplantarkeratosen. Hautarzt 1977;28:395-403.
4. Voigtländer V, Schnyder UW. Palmoplantarkeratosen. In: Korting GW, ed. Dermatologie in Praxis und Klinik. Stuttgart: Thieme Verlag, 1980: chap. 21, pp. 26-36.
5. Salamon T. An attempt at classification of inherited disorders of keratinization localized mainly, not exclusively on the palms and soles. Dermatol Monatsschr 1986;172:601-605.
6. Thost A. Über erbliche Ichthyosis palmaris et plantaris cornea; Inaug Diss. Heidelberg, 1880.
7. Unna PG. Über das Keratoma palmare et plantare hereditarium. Eine Studie zur Kerato-Nosologie. Arch Dermatol Syph (Berlin) 1883;15:231-270.
8. Hamm H, Happle R, Buterfass T, Traupe H. Epidermolytic palmoplantar keratoderma of Vörner: Is it the most frequent type of hereditary palmoplantar keratoderma. Dermatologica 1988;177:138-145.

9. Küster W, Becker A. Indication for the identity of Palmoplantar Keratoderma Type Unna-Thost with Type Vörner. Thost's family revisited 110 years later. Acta Derm Venereol (Stockh) 1992;72:120-122.
10. Greither A. Keratosis extremitatum hereditaria progrediens mit dominantem Erbgang. Hautarzt 1952;3:198-203.
11. Grin E, Salamon T, Milicevic M. Keratosis palmo-plantaris progrediens hereditaria mit dominantem Erbgang. Arch Klin Exp Dermatol 1962;214:378-93.
12. Rook AJ. Progressive palmoplantar keratodermia. Greither's syndrome. Br J Dermatol 1967;79:302.
13. Hübner U, Ziegenbalg W. Keratosis extremitatum hereditaria transgrediens et progrediens dominans. Derm Mschr 1969;155:340-3.
14. Van de Kerkhof PCM, Mali-Gerrits M, Ruiter D, Schuurman Stekhoven J. Keratosis palmoplantaris transgrediens et progrediens Greither. Br J Dermatol 1989;120:125-38.
15. Vörner H. Zur Kenntnis des Keratoma hereditarium palmare et plantare. Arch Dermatol Syph (Berlin) 1901;56:3-31.
16. Requena L, Schoendorff, Sanchez Yus E. Hereditary epidermolytic palmo-plantar keratoderma (Vörner type)-report of a family and review of the literature. Clin Exp Dermatol 1991;16:383-388.
17. Wachters DHJ, Frensdorf EL, Hausman R, et al. Keratosis palmoplantaris nummularis ('hereditary painful callosities'). Clinical and histopathologic aspects. J Am Acad Dermatol 1983;9:204-209.
18. Sybert VP, Dale BA, Holbrook KA. Palmar-plantar keratoderma. A clinical, ultrastructural and biochemical study. J Am Acad Dermatol 1988;18:75-86.
19. Hovorka O, Ehlers E. Meledakrankheit. Arch Derm Syph 1896;34:51.
20. Kogoj F. Die Krankheit von Mljet (mal de Meleda). Acta derm venereol Stockh 1934;15:264-269.
21. Bosnjakovic S. Vererbungsverhältnisse bei der sog. Krankheit von Mljet (mal de Meleda). Acta derm venereol Stockh 1938;19:88.
22. Degos MMR, Delort J, Charlas J. Ainhum avec kératodermie palmo-plantaire. Bull Soc fr Derm Syph 1963;70:136-138.
23. Miani G, Rasdoni L. Strozzamenti anulari e amputazioni spontanee della dita nelle keratodermie. Arch ital Derm Vener 1952;25:23-30.
24. Niles HD, Klumpp MM. Mal de Meleda. Review of the literature and report of four cases. Arch Dermatol Syphilol 1939;409-21.
25. Brambilla L, Pigatto PD, Boneschi V, Altomare GF, Finzi AF. Unusual Cases of Meleda Keratoderma Treated with Aromatic Retinoid Etretinate. Dermatologica 1984;168:283-286.
26. Gamborg Nielsen P. Two different clinical and genetic forms of hereditary palmoplantar keratoderma in the northernmost county of Sweden. Clin Genet 1985;28:361-366.
27. Gamborg Nielsen P. Mutilating palmoplantar keratoderma. Acta Derm Venereol (Stockh) 1983;63:365-367.
28. Kastl L, Anton-Lamprecht, Gamborg Nielsen P. Hereditary palmoplantar keratosis of the Gamborg Nielsen type. Clinical and ultrastructural characteristics of a new type of autosomal recessive palmoplantar keratosis. Arch Dermatol Res 1990;282:363-370.
29. Nesbitt LT, Rotschild H, Ichinose H, Stein W, Levy L. Acral keratoderma. Arch Dermatol 1975;111:763-768.

30. Vohwinkel KH. Keratoderma hereditarium mutilans. Arch Dermatol Syphil 1929;158:354-364.
31. Wirz F. Keratoma hereditarium mutilans: Zu dem gleichnämigen Aufsatz von Dr. KH Vohwinkel. Arch Dermatol Syphil 1930;159:311 312.
32. Greschebin S. Observations on ainhum: Does it exist as an independent disease? Urol Cutan Rev 1936;40:98-102.
33. Drummond A. A case od unusual skin lesions. Ir J Med Sci 1939:85-89.
34. Piers F. Hereditary keratodermia and ainhum. Br J Dermatol 1967;79:693-698.
35. Gibbs RC, Frank SB. Keratoma hereditaria mutilans (Vohwinkel): Differentiating features of conditions with constriction of digits. Arch Dermatol 1966;94:619-625.
36. Sierra JO, Blesa G, Montero E. Syndrome de Vohwinkel: Etude de quatre cas. Ann Dermatol Syphil 1975;102:41-45.
37. Chang Sing Pang AFI, Oranje AP, Vuzevki VD, Stolz E. Successful treatment of keratoderma hereditaria mutilans with an aromatic retinoid. Arch Dermatol 1981;117:225-228.
38. Ruiz-Maldonado R, Larano-Ferral N. Mutilating palmo-plantar hyperkeratosis with alopecia and erythematous inguinal and perianal lesions. Int J Dermatol 1972;11:31.
39. Camisa C, Rossana C. Variant of keratoderma hereditaria mutilans (Vohwinkel's syndrome). Treatment with orally administered isotretinoin. Arch Dermatol 1984;120:1323-1328.
40. Howel-Evans W, Mc Connell RB, Clarke CA et al. Carcinoma of the oesophagus with keratosis palmaris et plantaris (tylosis): a study of two families. Quart J Med New Ser 1958;27:413-29
41. Yesudian P, Premalatha S, Thambiah AS. Genetic tylosis with malignancy: a study of a South Indian pedigree. Br J Dermatol 1980;102:597-600
42. Parnell DD, Johnson SAM. Tylosis Palmaris et Plantaris. Its occurrence with internal malignancy. Arch Derm 1969;100:7-9
43. Riddick L, Brodkin RH, Gibbs RC. Palmar and plantar keratoderma with hyperpigmentation and gynaecomastia. Report of a case associated with primary adenocarcinoma of the lung. Acta Dermatovener (Stockholm) 1971;51:69-72.
44. Kerdel FA, Mac Donald DM. Palmo-plantar keratoderma associated with carcinoma of the bronchus. Acta Dermatovener (Stockholm) 1981;62:178-180
45. Votion V, Mineur P, Mirgaux M, Aupaix M. Hyperkératose palmo-plantaire associée a un adénocarcinome gastrique. Dermatologica 1982;165:660-663
46. Hillion B, Le Bozec P, Moulonguet-Michau I, Blanchet-Bardon C, Petit A, Stephan J, Civatte J. Hyperkératose palmo-plantaire filiforme et cancer du sein. Ann Dermatol Venereol 1990;117:834-836
47. Huriez CL, Agache P, Bombart M, et al. Epitheliomas spinocellulaires sur atrophie cutanée congénitale dans deux familles à morbidité cancéreuse élevée. Bull Soc Fr Derm Syph 1963;70:24-28.
48. Huriez CL, Agache P, Souillart F, et al. Scléroatrophie familiale des extrémités avec dégénérescence cellulaires multiples. Bull Soc Fr Derm Syph 1963;70:743-4.
49. Huriez CL, Deminati M, Agache P, et al. Génodermatose scléro-atrophiante et kératodermique des extrémités. Ann Dermatol Syphiligr 1969;96:135-46.
50. Lambert D, Planche H, Chapuis JL. La génodermatose scléro-atrophiante et kératodermique des extrémités. Ann Dermatol Vénéréol 1977;104:654-7.
51. Patrizi A, Lernia VD, Patrone P. Palmoplantar keratoderma with sclerodactyly (Huriez syndrome). J Am Acad Dermatol 1992;26:855-7.

52. Clouston HR. A hereditary ectodermal dystrophy. Can Med Assoc J 1929;21:18-31.
53. Clouston HR. Hereditary ectodermal dystrophy. Can Med Assoc J 1939;40:1-7.
54. Wilkey WD, Stevenson GH. A family with inherited ectodermal dystrophy. Can Med Assoc J 1945;53:226-230.
55. Williams M, Fraser FC. Hidrotic ectodermal dysplasia: Clouston's family revisited. Can Med Assoc J 1967;96:36-39.
56. Rajagopalan K, Hai Tay C. Hidrotic ectodermal dysplasia. Arch Dermatol 1977;113:481-485.
57. Patel R-RA, Bixler D, Norins AL. Clouston syndrome: A rare autosomal dominant trait with palmoplantar hyperkeratosis and alopecia. J Craniofac Gen Dev Biol 1991;11:176-179.
58. Feinmesser M, Zelig S. Congenital deafness associated with onychodystrophy. Arch Otolaryngol 1961;74:507-508.
59. Robinson GC, Miller JR, Bensimon JR. Familial ectodermal dysplasia with sensorineural deafness and other anomalies. Pediatrics 1962;30:797-802.
60. Schinzel A. A case of multiple skeletal anomalies, ectodermal dysplasia and severe growth and mental retardation. Helv Paediatr Acta 1980;35:243-251.
61. McKusick VA. Mendalian inheritance in man. 9th edition, Baltimore: The Johns Hopkins University Press, 1990.
62. Sharland M, Bleach NR, Goberdhan PD, Patton MA. Autosomal dominant palmoplantar hyperkeratosis and sensorineural deafness in three generations. J Med Genet 1992;29:50-52.
63. Bititci OO. Familial hereditary progressive sensorineural hearing loss with keratosis palmaris et plantaris. J Laryngol 1975;89:1143-6.
64. Hatamochi A, Nakagawa S, Ueki H, Miyoshi K, Inchi I, Konogsmark BW. Hereditary deafness in man. N Engl J Med 1969;281:713-20.
65. Olmsted HC. Keratoderma palmaris et plantaris congenitalis. Am J Dis Child 1927;33:757-64.
66. Costa OG. In: Acrokeratoses. University of Minas Geraes, Brazil: Belo Horizonte, 1962.
67. Poulin Y, Perry HO, Muller SA. Olmsted syndrome-congenital palmoplantar and periorificial keratoderma. J Am Acad Dermatol 1984;10:600-10.
68. Atherton DJ, Sutton C, Jones BM. Mutilating palmoplantar keratoderma with periorificial keratotic plaques (Olmsted syndrome). Br J Dermatol 1990;122:245-252.
69. Judge MR, Misch K, Wright P, Harper J. Palmoplantar and perioroficial keratoderma with corneal epithelial dysplasia: a new syndrome. Br J Dermatol 1991;125:186-188.
70. Papillon MM, Lefèvre P. Deux cas de keratodermie palmaris et plantire symetrique familiale (Maladie de Meleda) chez le frere et la soer. Coexistance dans le deux cas d'alterations dentaires graves. Societé Francais de Dermatologie et de Syphaligraphie 1924;31:82
71. Bork K, Löst C. Extrapalmoplantare Hautsymptome und weitere klinische und ätiologische, insbesondere immunologische Gesichtspunkte beim Papillon-Lefèvre-Syndrom. Hautarzt 1980;31:179-83.
72. Bach JN, Levan NE. Papillon-Lefèvre syndrome. Arch Dermatol 1968;97:154-8.
73. Haneke E. The Papillon-Lefèvre syndrome: keratosis palmoplantaris with periodontopathy: report of a case and review of the cases in the literature. Hum Genet 1979;51:1-35.
74. Bergman R, Friedman-Birnbaum R. Papillon-Lefèvre syndrome: a study of the long-term clinical course of recurrent pyogenic infections and the effects of etretinate treatment. Br J Dermatol 1988;119:731-736.
75. Van Dyke TE, Taubman MA, Ebersole JL et al. The Papillon-Lefèvre syndrome: neutrophil dysfunction with severe periodontal disease. Clin Immunol Immunopathol 1984;31:419-29.

76. Levo Y, Wollner S, Hacham-Zadeh S. Immunological study of patients with the Papillon-Lefèvre syndrome. Clin Exp Immunol 1980;40:407-10.
77. Stadler JF, Torres M, Taraud D et al. Anomalies fonctionelles des polynucléairs dans la maladie de Papillon-Lefèvre: trois observations. Nouv Presse Med 1982;11:2135-8.
78. Shams el Din A, Benton FR, Bottomley WK et al. Hyperkeratosis,periodontosis and chronic pyogenic infections in a 15-year-old boy. Ann Allergy 1984;53:11-14,55-6.
79. Shams el Din A, Benton FR, Bottomley WK et al. Hyperkeratosis,periodontosis and chronic pyogenic infections in a 15-year-old boy. Ann Allergy 1984;53:11-14,55-6.
80. Borroni G, Pagani A, Carcaterra A et al. Immunological alterations in a case of Papillon-Lefèvre syndrome with recurrent cutaneous infections. Dermatologica 1985;170:27-30.
81. Lyberg T. Immunological and metabolic studies in two siblings with Papillon-Lefèvre syndrome. J Peridont Res 1982;17:563-8.
82. Schroeder HE, Segar RA, Keller HU, Rateitschalk-Plüss EM. Behaviour of neutrophilic granulocytes in a case of Papillon-Lefèvre syndrome. J Clin Periodontol 1983;10:618-35.
83. Haim S, Munk J. Keratosis palmo-plantaris congenita, with periodontosis, arachnodactyly and peculiar deformity of the terminal phalanges. Br J Dermatol 1965;77:42.
84. Smith P, Rosenzweig KA. Seven cases of Papillon Lefèvre syndrome. Periodontics 1967;5:42.
85. Puliyel JM, Sridharan Iyer KS. A syndrome of keratosis palmo-plantaris congenita, pes planus, onychogryphosis, periodontosis, arachnodactyly and a peculiar acro-osteolysis. Br J Dermatol 1986;115:243-248.
86. Bureau Y, Horeau M, Barrière H, Dufaye C. Deux observations de doigts en baguettes de tambors avec hyperkératose palmoplantaire et lésions osseuses. Bull Soc Fr Dermatol Syph 1958;65:328-330
87. Bureau Y, Barrière H, Thomas M. Hippocratisme digital congénital avec hyperkératose palmo-plantaire et troubles osseux. Ann Derm Syph 1959;86:611-622
88. Hedstrand H, Berglund G, Werner I. Keratodermia palmaris et plantaris with cubbing and skeletal deformity of the terminal phalanges of the hands and feet. Acta Derm Venereol (Stockh) 1972;52:278-280
89. Koch HJ, Hübner U, Schaarschmidt, Thiel W. Keratosis palmoplantaris mit Trommelschlegelfingern, Hypotrichose, Hypohidrose und Zahndysplasie. Hautarzt 1991;42:399-401
90. Fuhs H. Zur Kenntniss der herdweisen Keratosen an Händen und Füssen. Acta Derm Venereol (Stockh) 1924;5:11-58.
91. Brünauer SR. Zur Symptomatologie und Histologie der kongenitalen Dyskeratosen. Dermatol Ztschr 1925;42:6-26.
92. Siemens HW. Keratosis palmo-plantaris striata. Arch Derm Syph (Berl) 1929;157:392-408.
93. Wachters DHJ. Over de verschillende morphologische vormen van de keratosis palmoplantaris, in het bijzonder over de "keratosis palmoplantaris varians". Thesis, Leiden, The Netherlands 1963:49-67.
94. Baes H, de Beukelaar L, Wachters D. Kératodermie palmoplantaire variante. Ann Dermatol Syph 1969;96:45-50.
95. Schnyder UW, Klunker W. Erbliche Verhornungsstörungen der Haut. Hdb d Haut u Geschl Kr 1966;7:908.
96. Bologa EI. Ein Fall van plantarer, inselförmiger Keratose in 5 Generatiuonen mit dominanten Erbgang. Hautarzt 1965;16:231.

97. Samberger F. Zur Pathologie der Hyperkeratosen. Arch Derm Syph (Berl) 1905;77:173-190.
98. Bloom D. Keratosis palmaris et plantaris hereditaria (occuring in four generations). Arch Dermatol 1932;25:1123.
99. Gasser I. Über atypische Palmo-Plantar-Keratosen. Dermatol Wchnschr 1950;121:289-296.
100. Dinger R. Untersuchungen über das Vorkommen von Palmoplantar-keratosen bei den Patienten der Hautklinik Hamburg-Eppendorf. Thesis, Hamburg 1964:44-45.
101. Roth W, Penneys NS, Fawcett N. Hereditary painful callosities. Arch Dermatol 1978;114:591-592.
102. Wachters DHJ, Frensdorf EL, Hausman R, van Dijk E. Keratosis palmoplantaris nummularis ("hereditary painful callosities"). J Am Acad Dermatol 1983;9:204-209.
103. Nogita T, Furue M, Nakagawa H, Ishibashi Y. Keratosis palmoplantaris nummularis. J Am Acad Dermatol 1991;25:113-114.
104. Hanhart E. Neue Sonderformen von Keratosis palmo-plantaris, u a eine regelmässig-dominante Form mit systematisierten Lipomen, ferner zwei einfach rezessive, mit Schwachsinn und z. T. mit Hornhautveränderungen des Auges (Ektodermalsyndrom). Dermatologica 1947;94:286-308.
105. Richner H. Hornhautaffektionen bei Keratoma palmare et plantare hereditarium. Klin Monatsbl Augenheilkd 1938;100:580-588.
106. Goldsmith LA. Tyrosine-induced skin disease. Br J Dermatol 1978;98:119-123.
107. Goldsmith LA. Tyrosinaemia II. Arch Int Med 1985;145:1697-1700.
108. Larrègue M, de Giacomoni PH, Bressieux JM, Odievre M. Syndrome de Richner-Hanhart ou tyrosinose oculo-cutanee. Ann Dermatol Vénéréol 1979;106:53-62.
109. Goldsmith LA, Kang E, Bienfang DC, Jimbow K, Gerald R, Baden HP. Tyrosinaemia with plantar and palmar keratosis and keratitis. J Paed 1973;83:798-805.
110. Hunziker N. Richner-Hanhart Syndrome and tyrosinaemia type II. Dermatologica 1980;160:180-189.
111. Rehak A, Selim MM, Yadav G. Richner-Hanhart Syndrome (tyrosinaemia-II). Br J Dermatol 1981;104:469-475.
112. Bienfang DC, Kuwabara T, Pueschel SM. The Richner-Hanhart syndrome: report of a case associated with tyrosinaemia. Arch Ophtalmol 1976;94:1133-7
113. Fraser NG, Mac Donald J Griffths WAD, Mc Phie JL. Tyrosinaemia type II (Richner-Hanhart syndrome) - report of two cases treated with etretinate. Clin Exp Dermatol 1987;12:440-443.
114. Fellman JH, Vanbellinghen PJ, Jones RT, Koler RD. Soluble and mitochondrial forms of tyrosine aminotransferase. Relationship to human tyrosinaemia. Biochemistry 1969;8:615-622.
115. Goldsmith LA, Thorpe J, Roe CR. Hepatic enzymes of tyrosine metabolism in tyrosinaemia II. J Invest Dermatol 1979;73:530-532.
116. Muller C. On the causes of congenital onychogryphosis. München Med Wchnschr 1904;49:2180-2.
117. Wilson AG. Three cases of hereditary hyperkeratosis of the nail bed. Br J Dermatol 1905;17:13-4.
118. Jadassohn J, Lewandowski F. Pachyonychia congenita. In: Jacobs Ikonographia Dermatologica. Berlin: Urban and Schwarzenberg 1906:29-31.
119. Kumer L, Loos HO. Congenital pachyonychia (Riehl type). Wien Klin Wochenschr 1935;48:174-8.

120. Schönfeld PHIR. The pachyonychia congenita syndrome. Acta Derm Venereol (Stockh) 1980;60:45-9.
121. Sivasundram A, Rajagopalan K, Sarojini T. Pachyonychia congenita. Int J Dermatol 1985;24:179-80.
122. Feinstein A, Friedman J, Schewach-Millet M. Pachyonychia congenita. J Am Acad Dermatol 1988;19:705-11.
123. Chang A, Lucker GPH, Kerkhof van de PCM, Steijlen PM. Pachyonychia congenita in the absence of other syndrome abnormalities. J Am Acad Dermatol, accepted for publication.
124. Haber RM, Rose TH. Autosomal recessive pachyonychia congenita. J Am Acad Dermatol 1988;19:705-11.
125. Paller AS, Moore JA, Scher R. Pachyonychia congenita tarda. A late-onset form of pachyonychia congenita. Arch Dermatol 1991;127:701-703.
126. Fred HL, Gieser RG, Berry WR, Eiband JM. Keratosis Palmaris et Plantaris. Arch Int Med 1964;113:866-871.
127. Raphael AL, Baer PN, Lee WB. Hyperkeratosis of Gingival and Plantar Surface. Periodontics 1968;6:118-120.
128. James P, Beggs D. Tylosis: A Case Report. Br J Oral Surg 1973;11:143-145.
129. Gorlin RJ. Focal Palmoplantar and Marginal Gingival Hyperkeratosis- A Syndrome. Birth Defects 1976;12:239-242.
130. Laskaris G, Vareltzidis A, Avgerinou G. Focal palmoplantar and oral mucosa hyperkeratosis syndrome: A report concerning five members of a family. Oral Surg 1980;50:250-253.
131. Jakac D, Wolf A. Papillomatös- verruköse Form der palmo-plantaren Keratodermie kombiniert mit anderen Anomalien der Verhornung sowie dysplastischen Zahnveränderungen. Hautarzt 1975;26:25-29.
132. Sakas EL, Gentry RH. Porokeratosis punctata palmaris et plantaris (punctate porokeratosis). Case report and literature review. J Am Acad Dermatol 1985;13:908-912.
133. Lestringant GG, Berge T. Porokeratosis punctata palmaris et plantaris. Arch Dermatol 1989;125:816-820.
134. Schiff BL, Hughes D. Palmoplantar keratosis acuminata with facial sebaceous hyperplasia. Arch Dermatol 1974;109:86-87.
135. Herman PS. Punctate porokeratotic keratoderma. Dermatologica 1973;147:206-213.
136. Friedman SJ, Herman PS, Pittelkow MR, Daniel WP. Punctate porokeratotic keratoderma. Arch Dermatol 1988;124:1678-1682.
137. Weiss RM, Rasmussen JE. Keratosis punctata of the palmar creases. Arch Dermatol 1980;116:669-671.
138. Powell FC, Venencie PY, Gordon H, Winkelmann RK. Keratoderma and spastic paralysis. Br J Dermatol 1983;109:589-596.
139. Nielsen PG. Punctate palmoplantar keratoderma associated with morbus Bechterew and HLA B 27. Acta Derm Venereol (Stockh) 1988;68:346-350.
140. Fegueux S, Crickx B, Perron J, Grossin M, Belaïch S. Hyperkératose palmo-plantaire filiforme et cancer recto-sigmoidien. Ann Dermatol Venereol 1988;115:1145-1146.
141. Bennion SD, Patterson JW. Keratosis punctata palmaris et plantaris and adenocarcinoma of the colon. J Am Acad Dermatol 1984;10:587-591.
142. Osman Y, Daly TJ, Don PC. Spiny keratoderma of the palms and soles. J Am Acad Dermatol 1992;26:879-81.

143. Lozinski AZ, Fisher BK, Walter JB et al. Metastatic squamous cell carcinoma in linear porokeratosis of Mibelli. J Am Acad Dermatol 1987;16:448-451.
144. Costa OG. Acrokeratoelastoidosis. Dermatologica 1953;107:164
145. Jung EG, Beil FU, Anton-Lamprecht I, Greten H, Nemetschek T. Akrokeratoelastoidosis. Hautarzt 1974;25:127-133.
146. Ritchie EB, Williams HM. Degenerative collagenous plaques of the hands. Arch Derm 1966;93:202-203.
147. Dowd PM, Harman RRM, Black MM. Focal acral hyperkeratosis. Br J Dermatol 1983;109:97-103.
148. Schöpf E, Schulz H-J, Passarge E. Syndrome of cystic eyelids, palmo-plantar keratosis, hypodontia and hypotrichosis as a possible autosomal recessive trait. Birth Defects 1971;8:219-221
149. Burket JM, Burket BJ, Burket DA. Eyelid cysts, hypodontia and hypotrichosis. J Am Acad Dermatol 1984;10:922-925.
150. Mehregan AH, Butler JD. A tumor of follicular infundibulum. Arch Dermatol 1961;83:78-81.
151. Happle R, Rampen FHJ. Multiple eyelid hidrocystoma syndrome: a new cancer syndrome? In: Clinical Dermatology - The CMD Case Collection. World Congress of Dermatology, Berlin, May 24-29, 1987. Ed: Wilkinson DS, Mascaró JM, Orfanos CE, Albers J. Uitg.: Schattauer, Stuttgart 1987:290-291.

SUMMARY, DISCUSSION AND CONCLUSIONS

At the end of the general introduction we defined two major objectives:

(i) The development of new therapeutical approaches for patients with ichthyosis and Darier's disease.

(ii) The development of a nosology for palmoplantar keratodermas and the exploration of new treatments.

Here we will integrate the observations described in this thesis with the existing protocols and we will construct an updated approach which will be applicable for office dermatology.

1. The development of a treatment program for patients with ichthyoses and Darier's disease.

The efficacy and limitation of the treatment of disorders of keratinization with topical moisturizing creams and/ or the systemic retinoid acitretin has been summarized in the general introduction. The side effects of acitretin and the limited efficacy of moisturizing creams require the development of new treatments for patients suffering from ichthyoses and Darier's disease. The topical retinoid 13-cis-retinoic acid (13-cis-RA), the vitamin D3 analogue calcipotriol and the cytochrome P-450 inhibitor liarozole proved to be challenging new approaches.

1.1. Comparative efficacy analysis

The efficacy of various treatments in various disorders is presented in Table I.

Acitretin, as systemic treatment, is effective in almost all disorders of keratinization. However, in patients with Darier's disease, erythrodermic lamellar ichthyosis and bullous ichthyosis, low-dose acitretin treatment is required as these disorders are characterized by acitretin induced aggravation. Acitretin still remains to be the golden standard with which new candidate drugs have to be compared and contrasted.

This thesis presents various alternatives for acitretin treatment.

Autosomal dominant ichthyosis vulgaris (ADIV), a disorder not severe enough to accept the side effects of systemic retinoids, in general responds well to topical moisturizing creams. ADIV did not improve unilaterally during topical treatment with 13-cis-retinoic acid (13-cis-RA) (part 1, chapter 1). **X-linked recessive ichthyosis (XRI)** usually only mildly improving with topical moisturizing creams, responds well to systemic acitretin. In this disorder topical 13-cis-RA, topical liarozole, and oral liarozole, also proved to be effective (part 1, chapters 1, 5, 6). In contrast, XRI did not improve on calcipotriol (own observations). **The isolated congenital ichthyoses non-erythrodermic lamellar ichthyosis (NELI), erythrodermic lamellar ichthyosis (ELI), ichthyosis**

Table I **Treatment efficacy comparison data derived from part 1 chapters 1-3 and 5-7, integrated with the existing approaches.**

		Treatment modalities					
		Topical moisturizing creams	Oral acitretin	Topical 13-cis-RA	Topical calcipotriol	Topical liarozole	Oral liarozole
Monogenic disorders of keratinization	ADIV	+		-			
	XRI	+/-	+	+	-	+	+
	NELI	-	+	+	+	+	+
	ELI	-	+	+	+		+
	CBIE	-	+	+	+	+	+
	IBS	-	+		-		
	SLS	-	+		+		
	CNS	-	-		-		
	Darier's disease	-	+	+/-	-		

ADIV autosomal dominant ichthyosis vulgaris
XRI X-linked recessive ichthyosis
NELI non-erythrodermic lamellar ichthyosis
ELI erythrodermic lamellar ichthyosis
CBIE congenital bullous ichthyotic erythroderma of Brocq
IBS ichthyosis bullosa Siemens
SLS Sjögren-Larsson syndrome
CNS Comèl-Netherton syndrome

bullosa Siemens (IBS), congenital bullous ichthyotic erythroderma (CBIE) and the **associated congenital ichthyosis Sjögren-Larsson syndrome (SLS)** demonstrated excellent responsiveness to oral acitretin, which so far was supposed to be the only effective treatment available. In this thesis, topical 13-cis-RA, topical calcipotriol, topical liarozole and oral liarozole revealed effective alternatives in the treatment of isolated congenital ichthyoses (part 1, chapters 1, 2, 5, 6). Whereas 13-cis-RA, topical and oral liarozole proved effective in the treatment of isolated congenital ichthyoses and XRI, calcipotriol had a more selective indication spectrum showing impressive improvement in some isolated congenital ichthyoses without a significant effect on XRI. However, a recent report suggesting that calcipotriol might be effective in ADIV and XRI, is at variance with our own observations (1); on the other hand recent reports on the failure of treatment of ADIV and XRI with calcitriol, suggest that both conditions are vitamin D3 resistant (2,3). Furthermore, a selective indication spectrum was observed in the calcipotriol treatment of associated congenital ichthyoses, revealing a good improvement in SLS, whereas **Comel-Netherton syndrome (CNS)** did not improve. **Darier's disease,** which is a classical indication for oral acitretin, proved to respond well to 13-cis-RA treatment, though a great interindividual variation was observed. Calcipotriol, however, did not constitute an alternative treatment for Darier's disease. In recent literature, the efficacy of 13-cis-RA in non erythrodermic lamellar ichthyosis (NELI) and Darier's disease has been reconfirmed (4,5).

1.2 Comparative side effect analysis

All-trans-retinoic acid has been shown to have some therapeutical effect in disorders of keratinization. However, irritation of the skin seriously limits the application of all-trans-retinoic acid. An important improvement was the development of **13-cis-Retinoic Acid (13-cis-RA)** formulations which proved to be far less irritative (part 1, chapters 1 and 7). However, 33% of the patients with ichthyoses and 43% of the patients with Darier's disease experienced some mild irritation of the skin which could be attributed to 13-cis-RA. In these patients the irritation could easily be controlled by reducing the application frequency. Therefore, 13-cis-RA can be regarded as a well tolerated topical treatment. **Topical calcipotriol** (part 1, chapters 2 and 3), was tolerated very well in patients inflicted with ichthyosis. Apart from some discomfort on both sides, attributable to the fatty ointment base, no unilateral side effects were reported. However, in Darier's disease pronounced irritation of the skin and aggravation of the disease were observed. Another drawback is that the maximal amount of calcipotriol ointment which is permitted to be applied per week is 100 gr. A major breakthrough in the topical treatment of ichthyoses is **liarozole** (part 1, chapter 6). Liarozole associated side effects (pruritus and irritation of the skin) were found in 42% of the patients, but did not constitute a major problem, with exception of 1 patient who experienced unacceptable irritation of the liarozole treated skin, and discontinued treatment. One patient prematurely stopped, because of adverse

effects equally at verum and placebo treated sides, which already started in the wash-out phase. In this patient it is likely that these adverse events were not due to the medication. Although we do not have data on a comparative study it is our impression that the use of calcipotriol, 13-cis-RA, and topical liarozole is associated with irritation of the skin, which in some patients may require discontinuation of treatment. Systemic treatment with liarozole (part 1, chapter 5) had a profile of side effects comparable with systemic acitretin treatment. As topical treatment with liarozole resulted in plasma liarozole levels substantially below the levels reached during systemic treatment, which implies that the topical application has far lower systemic availability in the treatment of disorders of keratinization. The fact that reduction of the daily dose in one patient from 300 to 150 mg. did not aggravate clinical symptoms suggests that the optimum daily dose of liarozole might be lower than 300 mg. Present studies with topical liarozole treatment, show that the application frequency of liarozole required for maintainance of clinical improvement, is considerably lower than the twice daily application used in our study (part 1, chapter 6). Alternatively it is attractive to speculate that the optimum concentration of topical liarozole might be lower than 5%. To minimalize side effects with oral and topical liarozole treatment, dose finding studies are indicated to establish the optimum treatment dose in ichthyoses.

1.3 Comparative cytometric analyses

Visual assessment of therapeutical efficacy of treatments in disorders of keratinization might be confused by the moisturizing capacity of topical treatment. The assessment of changes at the cellular level in vivo during treatment provides further information on therapeutic effects at the level of epidermal proliferation and differentiation and dermal inflammation. A selective action of retinoids is the induction of the keratins 4 and 13. In adult epidermis these keratins are normally not present. Therefore, the induction of keratins 4 and 13 during treatment, might be used to assess to what extent a treatment might have had a retinoid-mechanism as mode of action.

Treatment with 13-cis-RA (part 1, chapters 1 and 7) resulted in the induction of keratin 4 and 13 in patients with ichthyoses and Darier's disease. Furthermore, in patients with ichthyoses (part 1, chapter 1) induction of keratin 8 expression was observed after treatment with 13-cis-RA. Topical and systemic liarozole also induced keratins 4 and 13 (part 1, chapters 5 and 6) in parallel to the induction of these keratins by 13-cis-RA. In contrast, calcipotriol did not induce the expression of these keratins (part 1, chapter 4). From these observations we may conclude that 13-cis-RA and liarozole most likely exert their mode of action by a retinoid mechanism, in contrast to calcipotriol. However, this cell biological effect was not correlated with the clinical improvement. Therefore, the expression of these keratins can not be used as "technology assessment" for a "retinoid effect" in the individual patient.

Characterization of epidermal growth and differentiation was carried out using a panel of antibodies (part 1, chapters 1, 4-7). Although the treatments described in the chapters did improve the clinical aspects of the disorders of keratinization, no consistent pattern of modulation of these markers was observed. We conclude that the epidermal growth characteristics (Keratin 6, 16, Ki-67 binding and % SG2M phase), the differentiation characteristics (involucrin, keratin 10) and the inflammation markers (polymorphonuclear leucocytes, T-lymphocytes, B-lymphocytes, monocytes, macrophages and Langerhans cells) did not provide a method to precise the effect of the treatment in disorders of keratinization. However, an important limitation of the present methodology used for the flow cytometric assessment, might have been the biopsy method: razor blade biopsies. The collection of the material using this approach might not consistently have comprised the whole epidermis. Recently a method was developed to prepare pure epidermal cell suspension from punch biopsies. Further studies are required before we can refute the above mentioned parameters as useful in the assessment of cell biological effects during treatment of monogenic disorders of keratinization.

1.4 The therapeutical approach for today and tomorrow

Today, the treatment of autosomal dominant ichthyosis vulgaris (ADIV) consists of topical moisturizing creams. The topical retinoid 13-cis-RA is available in several European countries. In the Netherlands, topical 13-cis-RA is not available so far. Patients with X-linked recessive ichthyosis (XRI) can be treated with 13-cis-retinoic acid (13-cis-RA). The isolated congenital ichthyoses non-erythrodermic lamellar ichthyosis (NELI), erythrodermic lamellar ichthyosis (ELI), and congenital bullous ichthyotic erythroderma (CBIE) may be treated first with 13-cis-RA or calcipotriol. The treatment of choice in the associated congenital ichthyosis Sjögren-Larsson syndrome (SLS) is calcipotriol. In Darier's disease treatment with 13-cis-RA might be tried. With respect to calcipotriol it is important to realise that the maximum quantity of ointment, used per week, should not exceed 100 gr. With respect to topical 13-cis-RA no such upper limit can be given. However, a restrictive attitude is indicated for females at child bearing ages. Calcipotriol and 13-cis-RA are not indicated in females during pregnancy especially not in the first trimester. As 13-cis-RA or calcipotriol do not provide a treatment for the whole skin surface, these compounds may offer an elective treatment for the more severely involved or cosmetically disturbing areas. In case of an inefficient result of these treatments, systemic treatment with oral acitretin is the "ultimum refugium". Guidelines for the use of acitretin have been given in the thesis of van Dooren-Greebe.

Tomorrow, it is feasible that the treatment of monogenic disorders of keratinization will have been revolutionized by topical liarozole and as the next step systemic liarozole. In general, this compound proved to be well tolerated and very effective both as a topical and systemic treatment. Therefore, the future for patients with monogenic disorders of keratinization seems to be bright.

2. The management of palmoplantar keratodermas

2.1 The development of the nosology of hereditary palmoplantar keratodermas

In part 2 chapter 7 an updated classification is proposed offering the general dermatologist guidelines to classify the individual patient. As can be deducted from Table II, a patient visiting the dermatological department for the first time, can be classified in one of the 3 major groups "diffuse", "nummular/linear" or "papular" immediately, based on the clinical presentation. Subsequently, the mode of inheritance, palmoplantar hallmarks, histopathological examination, keratoses on distinct sites, and associated features provide additional information, eventually leading to the correct diagnosis.

2.2 The therapeutical approach

It has been reported by various groups that acitretin is highly effective in palmoplantar keratodermas. However, in patients having palmoplantar keratoderma with epidermolytic hyperkeratosis, acitretin aggravates the condition. As has been described in part 2 chapter 6, calcipotriol proved to fill this niche within the therapeutic management, as this compound proved to be effective in Vörners disease. Therefore, following histological classification of palmoplantar keratoderma, a treatment with acitretin or calcipotriol may be initiated. Based on the excellent results observed in the treatment of ichthyoses, it is attractive to speculate that liarozole might offer a new approach in the treatment of palmoplantar keratodermas.

The nosology and treatment of disorders of keratinization has changed by developments in molecular genetics and skin pharmacology. Further development of the nosology and new therapeutical principles will be realised probably before the end of this century.

REFERENCES

1. Kragballe K, Steijlen PM, Ibsen HH, van de Kerkhof PCM, Esmann J, Sorensen LH, Axelsen MB. Efficacy, tolerability and safety of calcipotriol ointment in disorders of keratinization. Results of a randomized, double-blind, vehicle-controlled, right-left comparative study. Arch Dermatol 1995;131:556-560.
2. Okano M. 1,25-(OH)2D3 on psoriasis and ichthyosis. Int J Dermatol 1991;30:62-64.
3. Okano M, Kitano Y, Yoshikawa K. A trial of oral 1-hydroxyvitamin D3 for ichthyosis. Dermatologica 1988;177:23.
4. Steijlen PM, Happle R, van Muijen GNP, van de Kerkhof PCM. Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. Dermatologica 1991;182:178-183.
5. Steijlen PM, Reifenschweiler DOH, Ramaekers FCS, van Muijen GNP, Happle R, Link M, Ruiter DJ, van de Kerkhof PCM. Topical treatment of ichthyosis and Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study. Arch Dermatol Res 1993;285:221-226.

Table II-a Classification of the hereditary palmoplantar keratodermas

Entity	Additional clinical hallmarks of hands/feet	Specific histology	Additional keratoses and associated features
Diffuse autosomal dominant palmoplantar keratodermas			
Unna-Thost	knuckle pads		keratoses on knees and elbows
Greither	transgreding progressive		
Vörner	knuckle pads	epidermolysis	
Sybert	transgreding progressive mutilating		
Vohwinkel	mutilating honeycombed nail defects		starfish-keratoses, alopecia, deafness, paraplegia, myopathy, ichthyosis
Howel/Evans			spinalioma of the oesophagus/palmoplantar skin
Huriez	sclerodactyly nail defects		malignant degeneration of atrofic skin
Clouston	papillomatous nail defects		hair defects, deafness, polydactyly, syndactyly, clubbing, mental retardation, dwarfism, photophobia, strabismus
Sharland			bilateral high frequency hearing loss
Olmsted	mutilating nail defects		periorificial keratoses, leucokeratosis, alopecia, joint hypermobility
Diffuse autosomal recessive palmoplantar keratodermas			
Mal de Meleda	transgreding progressive mutilating nail defects		perioral keratoses
Gamborg-Nielsen (Nagashima)	knuckle pads (mutilating) (transgreding)		
Acral keratoderma	linear lesions knuckle pads (mutilating)	dyskeratoses	linear keratoses on elbows, knees, ankles, Achilles tendon
Papillon-Lefevre	transgreding knuckle pads	vacuoles in corneocytes/ granulocytes reduced tonofilaments irregular keratohyalin granules	premature loss of both deciduous and permanent teeth, hyperkeratoses on knees, elbows, susceptibility to infections
Bureau-Barriere-Thomas	clubbing of fingers/toes		bone hypertrophy, thinning of the cortex of long bones, skeletal changes of terminal phalanges

Table II-b Classification of the hereditary palmoplantar keratodermas

Entity	Additional clinical hallmarks of hands/feet	Specific histology	Additional keratoses and associated features
Nummular/linear autosomal dominant palmoplantar keratodermas			
Wachters			woolly hair
PPK nummularis		local epidermolysis	
Richner-Hanhart		eosinophilic cytoplasmic inclusions in Malpighian cells	hyperkeratoses on elbows, knees, tongue, bilateral pseudoherpetic corneal ulcerations, corneal scarring, glaucoma, mental retardation
Pachyonychia congenita	nail defects (bullae)		follicular keratosis, oral leucokeratosis, (neo)natal teeth, steatocystoma multiplex, angular cheilosis, corneal dyskeratosis, cataracts, laryngeal lesions, hoarseness, mental retardation, hair anomalies, alopecia
Fred and Raphael	subungual and circumungual keratosis		oral mucosa hyperkeratosis
Nummular/linear autosomal recessive palmoplantar keratodermas			
Pachyonychia congenita Jakac-Wolf	nail defects (bullae) verrucous atrophic skin mutilating		see nummular/linear autosomal dominant palmoplantar keratodermas keratoses on knees, lower arms, buttocks, gingivitis, periodontitis
Papular autosomal dominant palmoplantar keratodermas			
Davies-Colley	(sometimes confined to the creases)	compact column of parakeratosis	spastic paralysis, ankylosing spondylitis, facial sebaceous hyperplasia, gastrointestinal malignancy
Acrokeratoelastoidosis	yellow papules palmoplantar margins	elastorrhexis	
Focal acral hyperkeratosis	palmoplantar margins negroid races		hyperkeratoses on the interphalangeal joints of fingers and toes
Hanhart			lipomata
Papular autosomal recessive palmoplantar keratodermas			
Schöpf-Schulz-Passarge			cystic eyelids, hypodontia, hypotrichosis, facial tumours of follicular infundibulum, malignant degeneration

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Op deze plaats zou ik eenieder willen bedanken die heeft bijgedragen aan het tot stand komen van dit proefschrift. Dit betekent automatisch dat ik alle medewerkers van zowel de polikliniek, kliniek, alsook het laboratorium bedank voor de prettige sfeer van deze afdeling, welke tot een ideale ambiance leidt voor zowel het uitoefenen van klinische als wetenschappelijke activiteiten, in dit proefschrift onlosmakelijk met elkaar verbonden. Dit brengt mij vanzelf tot mijn promotor en hoofd van de afdeling, Prof.dr.dr. P.C.M. van de Kerkhof, wiens verdienste het is om een groot aantal individuele persoonlijkheden, tot een geheel te smeden, dat enerzijds eendrachtig samenwerkt, zonder dit anderzijds van zijn individuele creativiteiten te ontdoen. Peter, ik wil je bedanken voor jouw aanstekelijk enthousiasme en steun, waardoor deze promotie tot stand is gekomen. Hiermee bedoel ik niet alleen je practische adviezen en stimulerende rol teneinde de voortgang te bespoedigen, maar ook je begrip in die tijd dat mijn gezinsomstandigheden dusdanig waren dat een noodgedwongen tijdelijke temporisatie onvermijdelijk was. Het is mij vooral in die periode gebleken hoe waardevol bovengenoemde ambiance op de afdeling was, waarbij ik eigenlijk iedere medewerker persoonlijk zou moeten noemen. Uiteraard wil ik nog mijn co-promotor Dr. P.M. Steijlen noemen, welke ik in het bijzonder waardeer als mens, waarbij principes stevig overeind blijven en tesamen met een uitgebreide kennis aangaande de (geno)dermatologie, leiden tot een accurate individuele patiëntgerichte benadering. Dankzij jouw uitspraak dat een proefschrift niet te snel moet worden voltooid, maar moet "rijpen" heb je bijgedragen aan een relaxatie welke een promotie alleen maar ten goede kan komen. Aangaande dit laatste, hebben voor mijn gevoel, promotor en co-promotor tesamen de ideale combinatie gevormd voor de totstandkoming van dit proefschrift. Wat betreft het immunohistochemische deel van dit proefschrift wil ik Lia Schalkwijk en Monique Link, afdeling pathologie alhier bedanken voor hun fraaie en snelle kleuringen. Betreffende het flowcytometrische deel wil ik graag Emiel Suijkerbuijk alsook Ivonne van Vlijmen en Candida van Hooijdonk danken. Diny de Heus bedank ik voor haar waardevolle adviezen aangaande de secretariële kant van deze dissertatie. Namens Janssen Pharmaceutica bedank ik Annie Heremans voor haar creatieve kwaliteiten waardoor liarozole beschikbaar is gekomen voor klinische trials binnen de dermatologie, Pierre Boegheim voor zijn prettige en structurele samenwerking, en Robert Wapenaar voor de statistische analyse van de liarozole studies. Uiteraard wil ik ook mijn ouders noemen welke mij een levensinstelling hebben meegegeven die niet alleen gericht is op mijzelf, maar die mij moreel verplicht ook open te staan voor anderen. Oprechte interesse vergemakkelijkt het verplaatsen in de ander en draagt zodoende bij tot een optimale patiëntenzorg. Last but not least wil ik mijn vrouw Els bedanken voor haar liefde jegens mij en Ceriel. Haar onbegrensde vertrouwen in iedereen leidt in deze tijd weliswaar soms tot frustratie, doch vormt een goede tegenbalans voor mijn soms te groot wantrouwen. Ceriel bedank ik voor zijn vrolijke aanwezigheid, welke in het voorbije jaar kortdurend twijfelachtig was en daarmee de bovenbeschreven temporisatie ten dele tot gevolg heeft gehad.

Curriculum Vitae

De schrijver van dit proefschrift, werd op 20-06-1965 te Maastricht geboren. Na het behalen van het eindexamen Atheneum B in 1983 aan het Sint Maartenscollege te Maastricht startte hij met de opleiding Geneeskunde aan de Rijksuniversiteit Limburg, waar hij in 1989 het artsexamen behaalde. In de periode van 01-10-89 tot 30-09-90 was hij werkzaam op de afdeling dermatologie van het Academisch Ziekenhuis Maastricht waar hij histopathologie van de huid bestudeerde, en wetenschappelijk onderzoek verrichtte op het gebied van allergologie en proctologie. Van 01-10-90 tot 14-09-92 vervulde hij een assistentschap dermatologie bij de maatschap Oostelijke Mijnstreek in het "De Wever Ziekenhuis" te Heerlen. Sinds 15-09-1992 is hij werkzaam op de afdeling dermatologie van het Academisch Ziekenhuis Nijmegen, waar hij onder leiding van Prof.dr.dr. P.C.M. van de Kerkhof en Dr. P.M. Steijlen werkte aan dit proefschrift. Sinds 01-12-1993 is hij in opleiding tot dermatoloog te Nijmegen (opleider Prof.dr.dr. P.C.M. van de Kerkhof). Hij is getrouwd met Els Evers en is sedert 16-01-1995 de trotse vader van Ceriel.

List of publications

1. Lucker GPH, Hulsmans R-F HJ, Kley van de AMJ, Staak van de WJBM. Contact-allergische reacties op Kathon CG in de periode 1987-1990. Ned T Dermatol Venereol 1991;1:44-46.
2. Lucker GPH, Hulsmans R-F HJ, Kley van de AMJ, Staak van de WJBM. Evaluation of the frequency of contact allergic reactions to Kathon CG in the Maastricht area - 1987-1990. Dermatology 1992;184:90-93.
3. Hulsmans R-F HJ, Lucker GPH, Kley van de AMJ, Groot de AC. Kathon CG und Methyldibromglutaronitril: wichtige Kontaktallergene bei Patiënten mit Perianalekzem. Ergebnisse einer retrospektiven Studie. Vasomed 1992;4:706-709.
4. Hulsmans R-F HJ, Lucker GPH, Kley van de AMJ, Groot de AC. Kathon CG and methyldibromoglutaronitrile: important contact allergens in patients with perianal dermatitis. In: Aktuelle Koloroktologie, band 9. Lux N, Hager Th (eds.). München: Edition Nymphenburg, 1992;9:275-286.
5. Lucker GPH, Koopman RJJ, Valk van de PGM. Millstone tattoo: a regional trade related dermatosis. Contactdermatitis 1993;29:217-218.
6. Steijlen PM, Lucker GPH. De neurocutane syndromen. Ned T Dermatol Venereol 1993;3:286-288.
7. Lucker GPH, Kerkhof van de PCM, Steijlen PM. Topical calcipotriol in the treatment of epidermolytic palmoplantar keratoderma of Vörner. Br J Dermatol 1994;130:543-545.
8. Lucker GPH, Steijlen PM. The coexistence of linear and giant porokeratosis. Dermatology 1994;189:78-80.
9. Chang A, Lucker GPH, Kerkhof van de PCM, Steijlen PM. Pachyonychia congenita in the abscence of other syndrome abnormalities. J Am Acad Dermatol 1994;30:1017-1018.
10. Lucker GPH, Koopman RJJ, Steijlen PM, Valk van der PGM. Treatment of palmoplantar lichen nitidus with acitretin. Br J Dermatol 1994;130:791-793.
11. Lucker GPH, Kerkhof van de PCM, Steijlen PM. The hereditary palmoplantar keratoses; an updated review and classification. Br J Dermatol 1994;131:1-14.
12. Lucker GPH, Steijlen PM. Widespread leukokeratosis in Noonan syndrome. Clin Exp Dermatol 1994;19:414-417.
13. Lucker GPH, Steijlen PM. The Olmsted syndrome; Mutilating palmoplantar and periorificial keratoderma. J Am Acad Dermatol, 1994;31:508-509.
14. Lucker GPH, Steijlen PM. De ziekte van Darier: een overzicht voor patiënten, hun familieleden en hulpverleners. Stichting Darier patiënten, 1994.
15. Lucker GPH, Kerkhof van de PCM, Steijlen PM. Effect of topical calcipotriol on congenital ichthyoses. Br J Dermatol 1994;131:546-550.
16. Lucker GPH, Steijlen PM. Oral liarozole treatment of hereditary ichthyoses: A clinical and immunohistochemical study (Abstr). J Invest Dermatol 1994;103:426.

17. Lucker GPH, Happle R, Steijlen PM. An unusual case of porokeratosis involving the natal cleft: porokeratosis ptychotropica?. Br J Dermatol 1995;132:150-171.
18. Lucker GPH, Steijlen PM. Pachyonychia congenita tarda. Clin Exp Dermatol 1995;20:226-229.
19. Lucker GPH, Steijlen PM. Acrokeratosis paraneoplastica (Bazex' syndrome) associated with acquired ichthyosis in Hodgkin lymphoma. Br J Dermatol 1995;133:322-325.
20. Lucker GPH, Kerkhof van de PCM, Cruysberg JRM, Kinderen der DJ, Steijlen PM. Topical treatment of Sjögren-Larsson syndrome with calcipotriol, a clinical study. Dermatology 1995;190:292-294.
21. Lucker GPH, van de Kerkhof PCM, Castelijns FACM, van Dijk MR, Steijlen PM. Topical treatment of ichthyoses with 13-cis-retinoic acid. A clinical and immunohistochemical study. Eur J Dermatol 1995;5:566-571.
22. Lucker GPH, Steijlen PM, Kerkhof van de PCM. Effect of topical calcipotriol on congenital ichthyoses. Br J Dermatol 1995;132:487.
23. Lucker GPH. De palmoplantaire keratodermiëen, een overzicht. Ned T Dermatol Venereol 1995;5:239-240.
24. Lucker GPH, Heremans A, Boegheim JPJ, Steijlen PM. Topical liarozole treatment of ichthyosis. A double-blind bilaterally paired, clinical and immunohistochemical study (Abstr). J Invest Dermatol 1995;105:496.
25. Lucker GPH. Is er een oorzaak en behandeling bekend voor hyperkeratosis palmaris et plantaris? Vademecum Perm Nasch Huisartsen 1995; jaargang 13 nr. 44.
26. Lucker GPH, Steijlen PM, Kragballe K, Kerkhof van de PCM. Flowcytometrical investigation of cell kinetics in monogenic disorders of keratinization before and after treatment with calcipotriol. Acta Derm Ven (Stockh) 1996;76:97-101.
27. Lucker GPH, Steijlen PM. Keratosis palmoplantaris varians et punctata. Klinische variabilität eines einzigen genetischen Defektes?. Hautarzt, accepted.
28. Lucker GPH, Heremans AMC, Boegheim PJ, Steijlen PM. Oral treatment of ichthyosis by the cytochrome P-450 inhibitor liarozole. Br J Dermatol, accepted.
29. Alkemade H, Lucker G, de Roos K-P, Steijlen PM, van de Kerkhof PCM. Systemische en locale bijwerkingen door locale corticosteroïd therapie van psoriasis. Ned T Dermatol Venereol, accepted.
30. Lucker GPH, van de Kerkhof PCM, Castelijns FACM, Steijlen PM. Topical treatment of Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study. J Dermatol Treat, accepted.
31. Lucker GPH, Heremans AMC, Boegheim PJ, Steijlen PM. Topical liarozole treatment of hereditary ichthyoses: A double-blind, placebo-controlled left-right comparative clinical and immunohistochemical study. submitted.
32. Kerkhof van de PCM, Gerritsen MJP, de Jong EMGJ, Lucker GPH, Steijlen PM. Indications for vitamin D 3 analogues beyond psoriasis vulgaris. submitted.

33. Feind-Koopmans A, Lucker GPH, van de Kerkhof PCM. Acquired ichthyosiform erythroderma and sarcoidosis. submitted
34. van de Kerkhof PCM, Lucker GPH, Steijlen PM, Heremans A. R-85355, a potent and selective 5-lipoxygenase inhibitor, fails to improve psoriasis. submitted.
35. Lucker GPH, Zeedijk N, Steijlen PM. The Huriez syndrome. Palmoplantar keratoderma with sclerodactyly. submitted.

STELLINGEN

behorend bij het proefschrift
"Ichthyosis, Darier's disease and Palmoplantar Keratoderma.
New insights in classification and therapy."

1. Classificatie van palmoplantaire keratodermieën is geen academische exercitie, doch van belang om de individuele patiënt adequaat te informeren omtrent het te verwachten beloop (progressie?, autoamputatie?), geassocieerde kenmerken (maligne degeneratie van de aangedane huid of de oesofagus?), en overerving.

2. De adequate classificatie van een palmoplantaire keratodermie, bepaalt het te volgen beleid aangaande aanvullende diagnostiek, periodieke controle en therapie.

3. Nauwkeurige observatie is elementair in de ontwikkeling van nieuwe geneesmiddelen. Liarozol, effectief in de behandeling van keratinisatiestoornissen, is als afgeleide van het antimycoticum ketoconazol, een goed voorbeeld hiervan.

4. De liganden voor de "steroid receptor superfamily" zijn met een zekere ziekte-specificiteit effectief bij de behandeling van monogene keratinizatiestoornissen.

5. Daivonex is een effectieve locale behandeling voor lamellaire ichthyosis, congenitale bulleuze ichthyosiforme erythrodermie, en het syndroom van Sjögren-Larsson.

6. Liarozol, een nieuwe endogene vitamine A afbraak remmer, is zowel in orale als in topische toedieningsvorm effectief in de behandeling van X-linked recessive ichthyosis, lamellaire ichthyosis, en congenitale bulleuze ichthyosiforme erythrodermie.

7. 13-cis-retinoic acid is in locale toedieningsvorm minder irritatief dan all-trans-retinoic acid en daardoor geschikt als therapie voor ichthyosis.

8. Geluk is relatief: gelukkig hij die ziet wat hij heeft; ongelukkig hij die ziet wat hij niet heeft.

9. Vorm en inhoud zijn vaak omgekeerd evenredig aan elkaar.

10. Evaluatie wordt soms verward met ego-massage.

11. Functioneringsgesprekscursussen zijn een moderne vorm van verborgen werkloosheid.

12. Tijd is geen snelweg tussen de wieg en het graf, maar ruimte om te parkeren in de zon. (Phil Bosmans)