13—*CIS*—Retinoic Acid in Rosacea

Clinical and Laboratory Findings

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Schmidt JB, Gebhart W, Raff M, Spona J. 13-CIS-retinoic acid in rosacea. Acta Derm Venereol (Stockh) 1984; 64: 15–21.

The paper presents clinical and laboratory data on 13-CIS-retinoic acid treatment in rosacea. Good-to-excellent results are reported for severe forms of rosacea. Side effects were tolerable and could easily be controlled. The role of sebaceous gland function was documented by both histological and skin sebum parameters. Diminution of sebaceous gland size and the decrease of skin sebum with alteration of single sebum fractions correspond with findings in acne. According to the findings the alteration of sebaceous gland function is one model of drug action in rosacea. Thus sebaceous gland dysfunction may play an important role in the pathogenesis of rosacea. *Key words: Rosacea; 13-CIS-retinoic acid therapy; Clinical findings; Skin surface lipids; Sebaceous gland histology.*
(Received April 30, 1983.)

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The excellent effect of 13-CIS-retinoic acid (Hoffman-La Roche) on severe forms of acne are well documented by both clinical and experimental data (1, 2, 3). Various effects of the drug are responsible for the impressive clinical improvement of the disease. In acne the suppression of sebaceous glands appears to play an essential role. This effect primarily was documented in the flank organ of the syrian hamster (4) and is also operative in human skin. In acne both the dose-dependent reduction of size of sebaceous glands (5) and the suppression and alteration of composition of skin surface lipids (6) correlate with clinical improvement. Recent investigations emphasize the correlation between sebum suppression and changes of the microbial population of sebaceous glands (7, 8). In addition retinoids modulate keratinisation (9) and display some anti-inflammatory effects (10).

The whole spectrum of these mechanisms confirmed for acne might also be responsible for the positive effect of 13-CIS-retinoic acid in the treatment of rosacea. Good to excellent results have been reported first by Nikolowski & Plewig (11, 12). Our own first clinical experience with the drug in rosacea confirmed the previous findings (13).

The purpose of the present study was to scrutinize the positive clinical efficiency by additional laboratory parameters reflecting the function of sebaceous glands.

PATIENTS AND METHODS

8 male and 5 female patients between 39 and 80 years of age with severe and often relapsing forms of rosacea were treated orally with 13-CIS-retinoic acid. The average duration of the disease before therapy had been 7 years. 8 patients had received oral tetracycline therapy (6 weeks to one year)—6 of them in combination with various topical preparations. 4 patients had performed local treatment only and one patient had no treatment before. The indication for 13-CIS-retinoic acid therapy was severity and long persistance of rosacea and lack of success or numerous relapses despite therapy. Independent of the body weight therapy was started with a dosage of 60 mg 13-CIS-retinoic acid daily and eventually reduced to 40 mg, depending on side effects.

Treatment duration was dependent on the success and varied between 6 to 20 weeks, the overall average duration of therapy being 16 weeks.

Clinical and serum controls (serumlipids, clotting parameters, liver parameters) were performed every two weeks. In 5 patients, biopsies were taken before and after therapy. Involved erythematous areas of the face were chosen and punched under local anesthesia. After discontinuation of the treatment, contralateral corresponding sites were biopsed and identically processed for routine histology using paraffin embedding, serial sectioning and hematoxylineosin-staining.

METHODS

Skin surface lipid investigations were performed every 4 weeks. Collection of skin surface lipids and analysis by thin-layer chromatography was performed by modifications of methods described by Weissmann (14) and Downing (15). Briefly, the skin surface is wiped for 15 seconds with a gauze saturated with 0.1% Triton X-100 to remove surface debris, desquamating epithelial cells and bacteria, rinsed with water, dried and then washed 15 seconds with a gauze saturated with hexane to remove skin surface lipids. Lipids for analysis are collected immediately and 2 hours after being cleaned by pipetting 2 ml of hexane from an automatic pipette into a glass cup with a 3.2 cm^2 area and scrubbed with a blunted teflon rod for 15 seconds.

Methyl nervonate serves as an internal standard permitting quantification of the lipid during subsequent thin layer chromatography. The internal standard also can be used for correction of any sample loss prior to spotting, since its loss would be proportional to the loss of extracted sebum.

Lipid samples are applied to analytical thin-layer chromatography (TLC) precoated plates LK 6 D obtained from Whatman Inc., Clifton, N.Y. 07014, USA. The thinlayer plates are developed three different solvents in 4% sulfuric acid for a couple for seconds. Subsequently the TLC plates are heated at 120°C for 1 hr, and scanned by a single beam spectrodensitometer (model CDS-200, Beckman Instruments, Inc., Irvine, Calif. 92713, USA).

An external standard is used to lacate cholesterol, free acid, triglycerides, methyl nervonate, wax esters, cholesteol esters and squalene. Methyl nervonate is used to calculate for recoveries and for quantitative calculation of various lipid fractions and sum of total lipids found as estimated by the integrator of the spectrodensitometer. The sensitivity limit of the assay for sebum production and skin lipids was 0.001 and 0.06 mg/3,2 cm^2, respectively. Data are given as mg lipid per 3.2 cm^2. All patients took part voluntary in the study and were informed about possible side effects.

Table I. *Clinical evaluation of 13-CIS-retinoic therapy in rosacea*

No. of pat.	Overall average daily dosage per kg body weight	Cumulation dose	Duration of treatment in weeks	Clinical status before/after therapy							
				Rhinophym		Papules		Pustules		Erythema	
Male											
1	0.5 mg	3.66 g	12	+++	+	+++	+	+	−	+++	−
2	0.8 mg	1.16 g	8	−	−	++++	−	++++	−	+	−
3	0.8 mg	4.50 g	16	+++	+	++++	−	−	−	+	±
4	0.8 mg	6.32 g	16	++	−	++++	−	+++	−	+	±
5	0.5 mg	4.48 g	12	++	±	++++	+	+++	−	++	+
6	0.8 mg	5.16 g	8	−	−	+++	−	++	−	++	+
7	0.5 mg	1.14 g	6	++++	++++	+++	+++	++	++	+	+
9	0.8 mg	3.10 g	6	−	−	++++	+	+	−	++	+
Female											
1	0.8 mg	7.00 g	20	−	−	++++	−	+	−	+	±
2	0.8 mg	2.60 g	8	−	−	+++	−	+	−	+	+
3	0.5 mg	3.46 g	8	−	−	++++	±	+	−	++++	+
4	1.0 mg	5.76 g	16	−	−	++++	−	++++	−	++++	+
5	0.8 mg	5.562 g	12	−	−	++++	−	±	−	+++	−

+ = minimal, ++ = mild, +++ = moderate, ++++ = severe.

RESULTS

Clinical

One male Patient discontinued therapy after 6 weeks because of lack of success. The others responded quickly and very effectively to therapy. After 2 weeks a reduction of pustular lesions became evident, followed by reduction of papules. After 4 to 6 weeks the improvement of the clinical status was already significant. The detailed results of clinical evaluations are given in Table I and are based upon our individual classification scheme reflecting the relative reduction of lesions. Those male patients who had rinophyma additionally registered diminution of size and a clinical impressive reduction of sebaceous gland hyperplasia.

No correlation could be found between severity of rosacea before treatment and onset of therapy effects or final success. In nearly all patients a minimal erythema still persisted, which usually disappeared some weeks after discontinuation of therapy. No significant influence of therapy on teleangiectasias was to be registered.

Histology

The most prominent finding in the biopsy material evaluated was a significant reduction of sebaceous gland size after therapy. All patients had received more than 5 g cumulative doses of 13-CIS-retinoid and—paralleling the planimetric results (5)—exhibited a diminuition of sebaceous gland size by more then 50% (Fig. 1 *a*, *b*). In addition, the ratio between undifferentiated and differentiated sebocytes changed dramatically during therapy, the latter being increased by 10-fold relative numbers (Fig. 2 *a*, *b*). Perifollicular fibrosis was observed at the periphery of nearly all shrunken sebaceous glands. The dermal connective tissue itself exhibited a reduction of it's overall linear thickness by 20%, since the average thickness of the whole dermis was diminished from 2.0 mm to 1.6 mm after treatment (Fig. 1 *a*, *b*), however a statistical significance could not be achieved due to the small numbers of patients.

Skin surface lipids

Former extended studies have shown the lack of significant discrepancies of skin sebum levels and sebum composition between rosacea patients and healthy controls (16, 17).

Table II. *Sebum production before and after one, two and three months of therapy with 13-CIS-retinoic acid*

Sebum production is expressed as replacement sum of lipids (mg/3.2 cm^2 area/2 hours)

No. of pat.	Sebum production			
	Pre-treatment	One month treatment	Two months	Three months
Male				
1	0.1747	0.0308	0	ND
2	0.2190	0.0047	ND	ND
4	0.1470	0.0300	0.081	0.058
5	0.0210	0	0	0
6	0.0720	0	0	0
Female				
3	0.0430	0.0290	0.024	0.030
5	0.1470	0	0	9

ND = not determined. Patient numbers are correlated with those in Table I

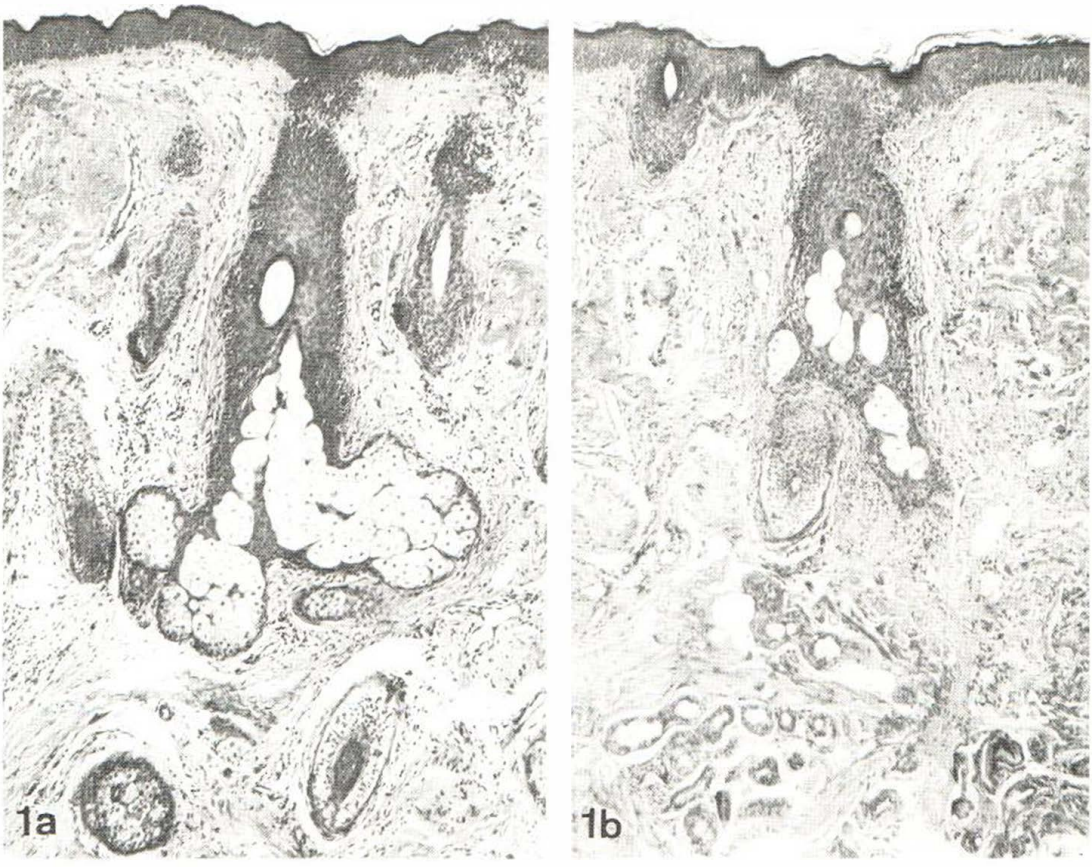


Fig. 1 a. Large sebaceous follicle before treatment with 13-CIS-retinoic acid. H. E., Orig. magn. ×65.

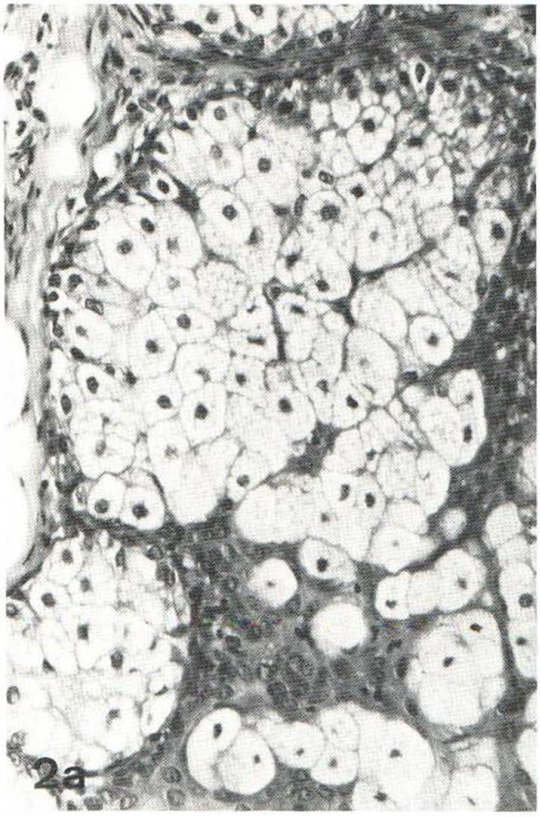
Fig. 1 b. Pronounced reduction of sebaceous gland size after treatment. Note also secretory coils of sweatglands in the lower portion of the micrography, indicating reduced thickness of dermal tissue. H. E., Orig. magn. ×65.

As for the present study the estimation of sebum production and determination of surface lipid composition was performed in 11 patients who were treated wtih 13-CIS-retinoic acid. After one month of treatment suppression of sebum production varied between 33 and 98% of pretreatment values in 4 patients. Four patients had pretreatment sebum production below the sensitivity of our assay, and 3 patients exhibited a reduction of sebum production to values below assay sensitivity within a month of treatment (Table II). Analysis of various lipid fractions revealed that colesterol esters were affected most by this treatment, and suppression of triglycerides was found to become increasingly suppressed during continuation of therapy. In addition, increased suppression of wax esters was also noted. But, no clear course of various fractions of lipids can be derived from the present data.

Side Effects

Clinical

Most of the patients (10) had cheilitis, some (4) complained about occassional dryness of the nasal mucous membrane. Occasionally pruritus in the affected skin was registered (6 patients). Mostly the same patients complained about occasional conjunctivitis. In all



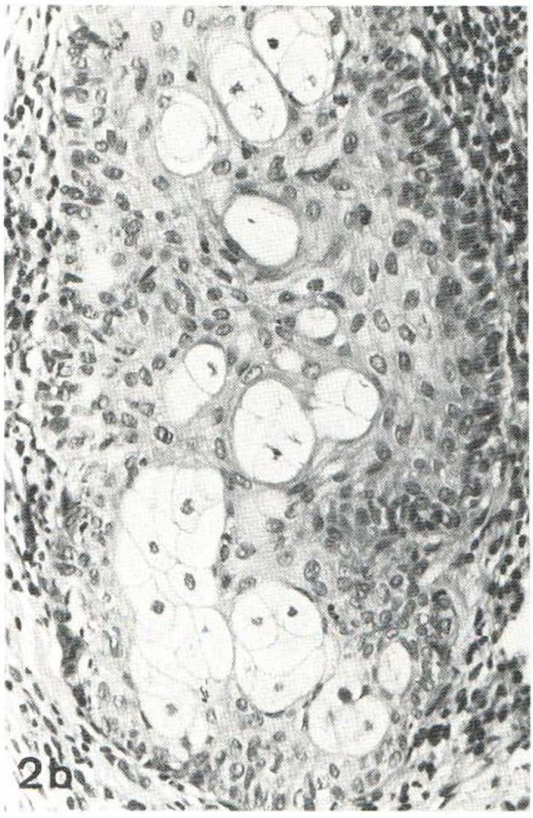


Fig. 2a. Mainly differentiated sebocytes before treatment. H. E., Orig. magn. ×250.

Fig. 2b. Only few differentiated, but many small undifferentiated sebocytes after treatment. H. E., Orig. magn. ×250.

cases side effects could be managed by modification of dosage and supporting cosmetic measures.

Laboratory

Elevation of serum lipids (cholesterol and triglycerides) was to be seen in 3 patients. All of them had elevated serum levels already before therapy. Those patients with serum levels within the normal range before therapy showed no increase into a pathologic range during therapy and the increase within the normal range was not significant. GOT enzyme increased during therapy in the one patient, who had slightly elevated liver enzymes already before, but γ-GT even sank in the same patient during therapy.

Post-Therapy Observation

3 female and 7 male patients are still on our observation. The female patients didn't show any relapse of rosacea one year (1 patient) up to 2 years (2 patients) after therapystop. 2 male patients with small cumulation doses (1.16 g, 1.14 g) showed relapses soon after therapystop—a fact which might indicate to a dose dependent duration of the remission period. But also 3 male patients with cumulation doses between 3.36 to 6.32 g 13-CIS-retinoic acid showed moderate mainly papulous recidives within one to 9 months. 2 male patients are free of lesions for two years after end of therapy.

DISCUSSION

The previously reported (11, 12, 13) excellent clinical results of 13-CIS-retinoic acid treatment in rosacea were confirmed by our present studies. Only severe, long lasting forms of the disease were treated and responded promptly with generally excellent results. However no final statement can be given concerning the remission period as 3 male patients with cumulation doses in a range which is known to suppress sebaceous gland function very effectively showed relapses within the first year after therapy. As far as can be said according to the few data female patients seem to show prolonged remission periods compared with males.

The cause for the discrepancy between the duration of remission periods in acne and rosacea might be that additional preexisting factors which contribute to the pathogenesis of rosacea like gastrointestinal disorders and elevated stimulability of the skin vessels are not influenced by the therapy.

The combination and persistance of those dysfunctions might contribute to the increased tendency towards relapses of the disease despite therapy.

Side effects with regard to skin and mucous membranes were moderate and could be controlled by dose-modification and/or skin care. Serum-lipids showed a moderate increase only in those patients who had elevated levels already before therapy. Also no elevation into pathological range could be found in liver enzymes but in one patient who had shown increased levels previously.

The present study confirms the importance of sebaceous gland suppression for rosacea among other mechanisms, because all patients but one (male patient No. 2) in whom sebum production was studied showed both reduction of sebum synthesis and decrease of number of skin lesions. Both the corresponding findings of histological diminution of sebaceous gland size and the decrease of total sebum (6) correlate withthe findings in acne. In all patients total sebum levels decreased rapidly during treatment. In patients with low initial sebum production the levels fell beyond assay sensitivity promptly. In others with initial seborrhoea the development of various sebum fractions became apparent: Cholesterol-esters seem to be affected most followed by suppression of triglycerides and wax esters.

Because of sebaceous gland suppression being one model of drug action in rosacea the conclusion might be drawn that dysfunction of sebaceous glands plays a more important role in the pathogenesis of the disease than assumed according to laboratory findings (16, 17).

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