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Treatment of facial actinic keratoses with a cream containing Octatrienoic acid: a multicenter clinical experience

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Abstract

BACKGROUND: Actinic keratosis (AKs) are premalignant skin lesions characterized by high rate of transformation in squamous cell carcinoma if not treated.

Pre-clinical published data on parrodiene-derivative 2,4,6-octatrienoic acid, encourages us to study and to evaluate the effect of a topical product containing it in patients affected by mild to moderate actinic keratosis.

METHODS: 70 subjects with at least 1-3 clinically diagnosed actinic keratosis lesions, non-hyperkeratotic, non-hypertrophic, localized on the face (I-II degree actinic keratosis) were enrolled in the study. The product was applied twice/day for 60 consecutive days.

RESULTS: After 60 days of treatment, a significant improvement in lesions occurred as shown by the decrease in the AKESA score ($p < 0.05$). Moreover, octatrienoic acid containing cream induced complete remission in 14 patients out of 70 (20%), ($p < 0.05$) and a complete clinical response in 63/70 patients (90%; $p < 0.5$; 95% confidence interval).

Compared to baseline, a significant number of patients reported improvement of each AKESA sub-score after 60 days of treatment: skin thickess improved in 46 patients ($p < 0.0001$), erythema in 21 patients ($p < 0.0001$) and atrophy in 57 patients ($p < 0.0348$).

The average pigmentation score significantly decreased from 1.50 to 0.79 ($p < 0.05$).

CONCLUSIONS: The results of the study, confirmed also by self-assessment, allow us not only to state that the use of topical octatrienoic acid was effective and well tolerated for topical treatment of AKs leading to overall clinical improvement in approximately 90% of subjects treated for 60 days.

Introduction

Actinic keratoses (AKs) are generally considered intra-epidermal skin lesions clinically classified as keratotic, atrophic, cornu cutaneum, verrucous, pigmented, and lichenoid variants even if there is not a universal accepted definition of AKs (1). They usually arise in photodamaged areas and, if left untreated, can develop into invasive squamous cell carcinoma. The rate of transformation to squamous cell carcinoma is not clear, but it is estimated to be between 0.1% and 10%, depending by number of lesions (i.e. people with more than 10 lesions present a probability of 14% within 5 years), with a lifetime risk of progression ranging from 6% to 10% (2; 3). The risk of metastasis of squamous cell carcinoma is estimated to be from 0.5% to 3.3% (4). AKs are mainly caused by non-ionizing radiation, particularly UV light during chronic sun exposure. The known risk factors for AKs include the following parameters: fair skin, male sex, age >50 years, sensitivity to the sun with reduced ability to tan and frequent sunburns, a high-fat diet, an immunosuppressed status, and genetic factors (2, 5-7). Human papilloma viruses act as co-carcinogens in the pathogenesis of AKs. The viral E6 protein of cutaneous human papillomaviruses interacts with the pro-apoptotic Bak protein, inhibiting the apoptosis (8, 9). AKs can appear as solitary lesions or can involve an entire field, such as photo-damaged areas (field of cancerization) (10). Genetic mutations are present both in AKs and in squamous cell carcinoma, confirming the malignant milieu of AKs. The

lesions showed a high mutation rate of the tumor suppressor gene *p53* and expression of telomerase (11,12). These initial alterations are often detectable under the microscope by the presence of hyperchromatic and pleomorphic nuclei with alteration of the nuclear cytoplasmic ratio, loss of polarity and frequent cellular superposition (13).

The primarily treatment of AKs includes lesion-directed option destruction or removal by means of physical treatment (14).

The most common treatment for solitary and thin AKs is liquid nitrogen freezing, which has a reported cure rate of between 75% and 99% (115); however, this is a cyto-destructive method that causes hypopigmented and unesthetic scars. Other current therapeutic approaches include curettage and electro- surgery, dermabrasion, laser and chemical peels, which are poorly tolerated by patients because of the high level of discomfort they cause.

Medical options for treating AKs are fluorouracil, retinoids, photodynamic therapy, imiquimod 5% cream, and diclofenac 3% in hyaluronic acid 2.5% gel. Fluorouracil is a topical antimetabolite useful for treating multiple and subclinical lesions, but a long period is often required to treat deep and hyperkeratotic AKs. The drug can cause wound infections, ulcers, and scarring, and has a reported clearance rate of 50% (16).

Systemic administration of etretinate reduces AKs in 85% of cases, whereas the topical application of retinaldehyde shows no therapeutic effect (17,18).

Photodynamic therapy involves the local application of a photosensitizing agent to AKs followed by exposure to light of a specific wavelength, which leads to cell death. The reported cure rate is between 69% and 93% (19).

Imiquimod 5% cream is effective in treating AKs, with a complete response reported in 45–57% of patients, although during treatment patients presented with adverse events ranging from redness to hemorrhagic crusted lesions (20).

Diclofenac 3% in hyaluronic acid 2.5% gel is a topical non-steroidal anti-inflammatory drug

(NSAID) formulation that offers an effective approach to treating AKs. It has a low incidence of adverse reactions and its mechanism of action is crucial in preventing the tumorigenesis of epithelial cells (21). The drug acts as an inhibitor of cyclo-oxygenase (COX)-2, favoring the block of prostaglandin E_2 synthesis involved in the suppression of T- and B-cell proliferation and the cytotoxic activity of natural killer cells (22). A complete healing of AKs with diclofenac 3% in hyaluronic acid 2.5% gel was seen in 50% of treated lesions (23).

Interesting clinical data have been published on Ingenol mebutate (24). Ingenol Mebutate, a macrocyclic diterpene ester, is the active agent in the sap of the plant *Euphorbia peplus*, which has long been used as a traditional remedy for common skin lesions, including cancerous lesions (25,26).

Preclinical studies have indicated that ingenol mebutate is a pleiotropic effector that induces rapid and direct cell death and immune responses mediated by specific activation of protein kinase C delta, including neutrophil-mediated oxidative burst and clearance of tumors (27,28, 29).

In the published (24) pooled analysis of the two trials involving the face and scalp, the rate of complete clearance was higher with ingenol mebutate than with placebo (42.2% vs. 3.7%, $P<0.001$). Local reactions peaked at day 4, with a mean maximum composite score of 9.1 on the local-skin-response scale (which ranges from 0 to 4 for six types of reaction, yielding a composite score of 0 to 24, with higher numbers indicating more severe reactions), rapidly decreased by day 8, and continued to decrease, approaching baseline scores by day 29. In a pooled analysis of the two trials involving the trunk and extremities, the rate of complete clearance was also higher with ingenol mebutate than with placebo (34.1% vs. 4.7%, $P<0.001$). Local skin reactions peaked between days 3 and 8 and declined rapidly, approaching baseline by day 29, with a mean maximum score of 6.8. Adverse events were generally mild to moderate in intensity and resolved without sequelae. Further clinical trials are needed to prove the applicability of this novel substance for the treatment of AK including the evaluation of efficacy and tolerability.

Many treatment modalities are available for the treatment of AK. Recent developments have focused on the management of the whole actinically damaged field. In this regard, several topical drugs have

been approved for AKs, differing in clearance rates, side effects, application and cost. Nevertheless, research is continuing aiming in the development of the "ideal" treatment of AKs which combines higher clearance rates with few side effects, short treatment duration and low costs.

Between medical available options the topical retinoids have a long history of use for the prevention and treatment of non-melanoma skin cancers (30). Of course also retinoid treatment is not free of side effect and during its use precautions are employed i.e. sun protection from sun light to avoid photodegradation.In the last years a new family of compounds, the psittacofulvins a class of pigments found in plumage of Ara macao got the researchers attention because of some characteristic similar to retinoids (31).

In particular, the parrodiene-derivative 2,4,6-octatrienoic acid, is characterized by a original mechanism of action respect to the action of Retinoic acid and this compound have a potential interest for the treatment of actinic lesions as:

• rapidly activates the gene involve in to the production of the enzyme proposed to his catabolism in derivative with earlier activity than classical retinoids (32, 33)

• activates PPARy pathway (34, 35)

• has anti-inflammatory action (36, 37)

• acts on the main markers of dermal remodeling (37)

To underline that PPARy plays an important role in the control of proliferation of keratinocytes and the epidermal differentiation. His expression is rapidly up-regulated following the changes that stimulate the proliferation of keratinocytes (hair growth and skin injury). The PPARy ligands are drug candidates for the treatment of epidermal conditions characterized by inflammation, hyper-proliferation of keratinocytes and psoriasis.

PPARy is, therefore, a potential target for the treatment of actinic keratosis.

On the basis of the Pre-clinical data on the octatrienoic acid, we carried out this study to evaluate the effect of the product containing it in patients affected by mild to moderate actinic keratosis.

Methods

Patients and Treatment

The following were included in the study: patients responding to the following Inclusion and Exclusion Criteria:

1. Male or female, aged ≥ 20 years.
2. The patient who agrees to enter the firm by signing the written informed consent.
3. Patient with at least 1-3 clinically diagnosed actinic keratosis lesions, non-hyperkeratotic, non-hypertrophic, localized on the face (I-II degree actinic keratosis).
4. The patient accepts to collect photographic material of the selected lesion and used as part of the data of the Study.
5. Patient in good general health
6. Informed consent

They were excluded from the study:

1. The patient with relapse of invasive squamous cell carcinoma (SCC).
2. The patient with reactive or secondary lymphadenopathy.
3. The patient under conditions of malnutrition or visibly poor.
4. The patient with skin conditions in the treatment area that can be exacerbated by treatment (eczematous and desquamating diseases).
5. The patient who currently uses or has used on the area to be treated retinol, corticosteroids, cryosurgery, curettage, 5-fluorouracil (5-FU), imiquimod, topical diclofenac, retinoids, prednisone / prednisolone or other topical treatment for actinic keratoses (which laser abrasion, ermoabrasion, chemical peeling) 28 days before the screening visit.
6. The patient who has practiced systemic chemotherapy for neoplasia or has taken immunosuppressants; Phototherapy was performed 6 months before the screening visit on the evaluation area.
7. The patient engaged in activities involving excessive or prolonged exposure to sunlight.
8. History of allergy or sensitivity to related compounds or other components of the product of the experimentation formulation.
9. Pregnant woman, breastfeeding (puerperium) or planning to become pregnant during the Study.

10. The patient who took any The patient who took any experimental drug in the 8 weeks prior to the recruitment visit.

This multicenter open-label pilot trial was performed in Italy. This study obtained the Ethical committee approval in each center and written informed consent was received by study participants. The study was conducted in accordance with ethical guidelines of the Declaration of Helsinki.

70 AKs patients were enrolled. Patients with suspected basal-cell or squamous-cell carcinoma were excluded as well if affected by other dermatologic disorders. Moreover, we excluded patients with a recent history of treatment with immunosuppressive/immunomodulating medications, cytotoxic drugs, ultraviolet B phototherapy, or oral retinoids.

The sample group was affected by multiple AKs in the face and/or in the scalp.

The cream product was self-applied twice daily for 60 consecutive days.

All patients were advised to avoid sun exposure.

Assessments

To assess the efficacy of the therapy, we evaluated the lesions clinically and by means of scoring system (AKESA), previously validated in an interventional clinical trial (38), based on the clinical presence of erythema, scaling/tickening, and atrophy on a target AK lesions. A numeric value from 0 to 3 was attributed to each AK clinical feature (baseline AKESA maximum 9) up to complete remission (disappearance of all features in the target lesion, AKESA endpoint 0).

An AKESA Score of 0, disappearance of all features in the target lesions, was considered a Complete Remission end-point; a >75 % improvement in the AKESA score constituted a Complete Clinical Response.

Statistical Analysis

The characteristics of the subjects, detected at the baseline visit, were presented by descriptive statistics (mean, DS, minimum and maximum values, or frequency tables, consistent with the type of data).

These values were compared, through Student's t-test or $\chi 2$-test, to verify the homogeneity of the data.

Statistical analysis of data will be performed in the following populations:

"Intention-to-Treat" population (ITT): All randomized patients with administration of a treatment dose and at least one evaluation of the main parameters after baseline visitRegarding the Performance parameters, the analysis was performed on the variations with respect to the baseline visit of the values obtained at the T3 visit; comparisons were made using Student's t-test paired or McNemar's Tes, consistent with the type of data to be analyzed and the normality / non-normality of data distribution, which was ascertained through the Shapiro-Wilk test.

On the continuous data distributed normally and detected at T1, T2 and T3, the analysis of the variance for repeated measurements was also carried out, to evaluate the trend over time of the analyzed variable.

Response to treatment was presented using frequencies and percentages at T2 and T3.

Results

70 AKs patients were enrolled, 1 drop out was reported due to treatment skin desquamation and itch, while 69 completed the study. 15 women and 54 men, with a mean age of 69 years participated to the study.

The demographic and anamnestic characteristics of the subjects included in the study detected at the baseline visit are summarizes in Table I.

As shown, 69 adult Caucasian subjects were included, with an average age of 69 (age between 54 and 82 years) of both sexes, with an average weight of 74.67 Kg.

Table I - Demographic information and Vital parameters at the initial visit (T1)____

Age (years)	
Subject number	69
Mean ± SE	69.73 ± 1.73

Sex	
Female	15 (22.22%)
Male	54 (77.77%)
Weight (Kg)	
Subject number	69
Mean ± SE	74.67 ± 3.34
Height (cm)	
Subject number	69
Mean ± SE	1.68 ± 1.52
Blood pressure (mmHg)	
Subject number	69
Systolic pressure	
Mean ± SE	127.78 ± 2.04
Diastolic pressure	
Mean ± SE	80.37 ± 2.43
Heart rate (bpm)	
Subject number	68
Mean ± SE	73.96 ± 0.85

After 60 days of treatment (Time-endpoint), there was a significant improvement of the lesions based on the decrease in mean AKESA score from baseline, passing from 3.27 to 1.33 ($p<0.05$, Wilcoxon Signed Rank Test) (Table II). Indeed, the treatment induced a Complete Remission in 14 of 70 patients (20%), ($p < 0.05$), and a Complete Clinical Response in 63/70 patients (90%) ($p < 0.5$; 95% confidence interval) (Table III).

Table II - Degree of severity of actinic keratoses to the final visit to 60 days (T3)

AKESA - total score	**N°**
0	14 (20.00%)
1	30 (42.85%)
2	15 (21.42%)
3	8 (11.42%)
4	2 (2.85%)
AKESA - total score - medium value -T3	
Subject number	69
Mean ± SE	1.33 ± 0.12
AKESA total score - variation vs T1	
Subject number	69
Mean ± SE	-1.93 ± 0.14 *

* $p < 0.05$ T3 vs T1 (variation)

Table III - Clinical Response evaluation by AKESA score

Total clearance (AKESA = 0)	
Yes	14 (20.00%)
No	55 (78.57%)
Partial healing (score of at least 75% of the high score at baseline)	
Yes	63 (90.00%)
No	7 (10.00%)
Stability condition (score less than 50% of the high score at baseline)	
Yes	37 (52.85%)
No	33 (47.14%)

Compared to baseline, a significant number of patients recorded an improvement of each AKESA sub-score after 60 days treatment: the skin scaling/thickening improved in 46 patients ($p < 0.0001$), erythema in 21 patients ($p < 0.0001$) and atrophy in 57 patients ($p = 0.0348$; McNemar's Test) (Table IV).

Table IV. Degree of severity of actinic keratoses to visit at 60 days (T3)

Erythema					
	N° pz %	Absent	Present	Total	
	Absent	1 1.45	0 0.00	1 1.45	
	Present	20 28.99	48 69.57	68 98.55	
	total	21 30.43	48 69.57	69 100.00	McNemar's Test : p < 0.0001
Scaling/thickening					
	N° pz %	Absent	Present	Total	
	Absent	18 26.99	0 0.00	18 26.09	
	Present	28 28.99	23 33.33	51 73.91	
	total	21 30.43	48 69.57	69 100.00	McNemar's Test : p < 0.0001
Atrophy					
	N° pz %	Absent	Present	Total	
	Absent	48	2	50	

		69.57	2.90	72.46	
	Present	9 13.04	10 14.49	19 27.54	
	total	57 82.61	12 17.39	69 100.00	McNemar's Test : p = 0.0348

Moreover, the average pigmentation score decreased from 1.50 to 0.79 ($p < 0.05$, Wilcoxon Signed Rank Test) (Table V).

Table V- Pigmentation variation during the study

	T1	T2	T3
Subject number Mean ± SE	70 1.50 ± 0.26	70 1.09 ± 0.19	70 0.79 ± 0.15*

* $p<0.05$ T3 vs. T1

Concerning the safety, 3 adverse events were registered during the study, in 3 patients: one (dry skin) has been reported as "Serious". The other two adverse events, reported as non serious, were "peeling" and "dry", both moderate as intensity.

The overall Performance judgment expressed by the investigators was Excellent in 55.2% of subjects (37), Good in 41.4% of subjects (32) and Sufficient in one subject (3.4%).
The opinions about efficacy and tolerability expressed by the patient and by the investigators at the end of the study (Final visit after 60 days) are summarized in Table VI.
The treatment efficacy opinions expressed by the patients were "Very" for 27% (20/69), "Good" for 46% (12/65) and "Moderate" for 27% (7/26) of patients, while those expressed by investigators were "Excellent", "Good" or "Moderate" for 19%, 65% and 15% respectively.
With regard to tolerability, for almost all patients the judgment was Good / Excellent tolerability (92% and 96% of the judgments respectively for patient and investigators) with a single patient who expressed a negative judgments.

Table VI – Efficacy and tolerability judgment by investigators and patients

	Moderate	Good	Excellent
Efficacy Patients Investigators	 19 (26.92%) 10 (15.38%)	 31 (46.15%) 45 (65.38%)	 19 (26.92%) 14 (19.23%)
	Sufficient	Good	Excellent
Tolerability Patients	 2 (6.89%)	 10 (34.48)	 17 (58.62%)

Investigators	1 (3.44%)	10 (34.48%)	18 (62.06%)

Discussion

AKs are a very frequent medical condition and represent a large health burden for the population that is even increasing in incidence. The natural course of AKs is not predictable and on clinical grounds it is not possible to determine which AK lesion will progress into invasive Squamous Cell Carcinoma (SCC).

Without treatment AK may progress into invasive SCC in about 5 - 10% of cases, but showing a greater risk of invasive growth in patients on chronic immunosuppression. At the present time, it is not possible to predict which lesion will progress and at which time point this may happen by either clinical or histological examination. Therefore treatment of all AK is recommended.

The development from AK to invasive SCC represents a continuous process and the presence of whole actinically damaged fields in these patients require treatment modalities that are able to clear all visible and ideally also non-visible subclinical lesions within the area of field cancerization.

Moreover, prevention strategies are of major importance. In this regard, sun protections by sunscreens as well as sun protection habits play a significant role.

Many treatment modalities are available for AK. These may be sub-divided into two major categories, namely lesion-directed treatments and field-directed treatments.

Lesion-directed therapy of AK includes surgical removal of single lesions by excision or curettage and physically destructive methods such as cryotherapy and laser treatment. Surgical excision is not used as a first line treatment for AK and is only performed if the lesion is highly suspicious for invasive SCC. Surgical treatments are invasive procedures which require local anesthesia, painful for the patients and result in scarring. However, superficial curettage of a lesion may be beneficial in hyperkeratotic lesions and in combination with field-directed treatment modalities that have been developed in the past decades considering the fact that AK arise in areas of field cancerization novel,

which may include treatment of the complete area of field cancerization, given that they are applied on a large surface.

Imiquimod was the first substance which showed "highlighting" of subclinical lesions during treatment and clearance of the lesions after cessation of therapy. However, this phenomenon may also be observed under treatment with diclofenac or PDT. Topical application of the long available substance 5-FU to a large area would also result in necrosis and inflammation of the treated area. But due to the non-selective mechanism of action of 5-FU it would also be associated with marked side effects including pain, erythema, crusting, pigmentary changes or even scarring.

All currently available drugs have their advantages and disadvantages resulting in different clearance rates, tolerability, treatment duration and costs.

In our study the use of the topical octatrienoic acid was effective and well tolerated for the topical treatment of AKs leading to a clinical improvement overall in about 90% of subjects treated for 60 days, without any side effects.

The treatment with octatrienoic acid cream, can be promising and quote for all those forms of AK in the initial phase and should be considered support to traditional therapies and thus can reduce the number of invasive treatments that can often create quite debilitating side effects. It could be recommended to the preparation of traditional therapies i.e. 2 months before, and to maintain post-therapeutic effect and in all patients who present a marked photoaging, to minimize the appearance of precancerous lesions. Future studies are needed to assess the benefits and safety of treating larger areas of skin and for a longer time.

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