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Evaluation of Ingenol mebutate efficacy for the treatment of actinic keratosis with Antera 3D camera

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Abstract. – OBJECTIVE: Cumulative exposure of the skin to ultraviolet radiation promotes mutation in keratinocytes and their abnormal growth led to the formation of scaly lesions, called actinic keratoses (AKs). Its incidence is growing at an emerging rate, becoming a worldwide problem especially for occupational ultraviolet (UV) rays exposure. Detectable lesions are often associated with field changes, where the surrounding skin is altered and subclinical lesions may be present. Thus, a field-directed therapy, such as topical treatment, should be preferred for the prevention of invasive cancer development. A retrospective analysis was made, evaluating the efficacy of ingenol-mebutate gel, using a novel device the 3D *in vivo* optical skin Imaging (Antera 3D, Miravex, Ireland).

PATIENTS AND METHODS: We included all patients with multiple non-hypertrophic Aks, to whom it was prescribed ingenol-mebutate gel, applied at the dosages of 0.015 for lesions in the scalp/face (for 3 consecutive days) and at the dosage of 0.05% for lesions in the trunk and/or extremities (for 2 consecutive days).

RESULTS: A reduction of the lesions and of median hemoglobin levels, after a follow-up of 60 days, was observed in 100% of patients.

CONCLUSIONS: Ingenol mebutate gel, the last topical molecule appeared in the Italian market showed its efficacy using Antera 3D also in terms of hemoglobin reduction. Therefore, this camera could be considered an useful tool for the identification of the area to be treated and for therapeutic follow-up.

Key Words:
Actinic keratosis, Antera 3D, Cancerization field, *Euphorbia pheplus*, Sun damage.

Introduction

Actinic Keratosis (AKs) are keratotic lesions occurring on chronically light-exposed adult skin, especially in fair-skinned people. They represent the earliest manifestation of squamous cell carcinoma (SCC), often considered *in situ* skin cancer, since they share histological and molecular aspects[1]. Normal-appearing skin, surrounding AKs, frequently expresses molecular changes in the form of p53 mutations. The whole area, therefore, has the potential to develop AK, a chronic condition in which new or recurrent lesions continue to develop over time, on a background of subclinical disease[1,2]. Ultraviolet (UV) radiations, considered completed carcinogens, leads to multiple genetic (and epigenetic) alterations, that promote preferential growth of altered keratinocytes, which may ultimately manifest as malignant evolution[3]. In young age, the body can repair some of the damage, over the time the damage accumulates and the body is less able to repair itself. The process commonly starts on large fields of skin exposed to UV radiation ("field cancerization") and if UV rays continue to hit the skin, AK or other skin cancers arise. It was observed that a patient with multiple Aks (>5) has an annual risk of developing invasive squamous cell carcinoma ranging up to 80%; 82.4% of SCC derived from previous AK and/or in close proximity, but none is able to predict which of them will progress[3-5]. They affect half of the global population and prevalence may vary with geographical location and age. The diagnosis of AK is typically made clinically, detectable only as an area of roughening on the skin surface. Patients often refer a history of relapsing, remitting lesions, therefore if left untreated they may turn into more invasive skin cancer. The new therapeutic approach is oriented to make an early diagnosis and to treat all lesions as earlier as possible. Many treatment modalities are available, although recent developments have

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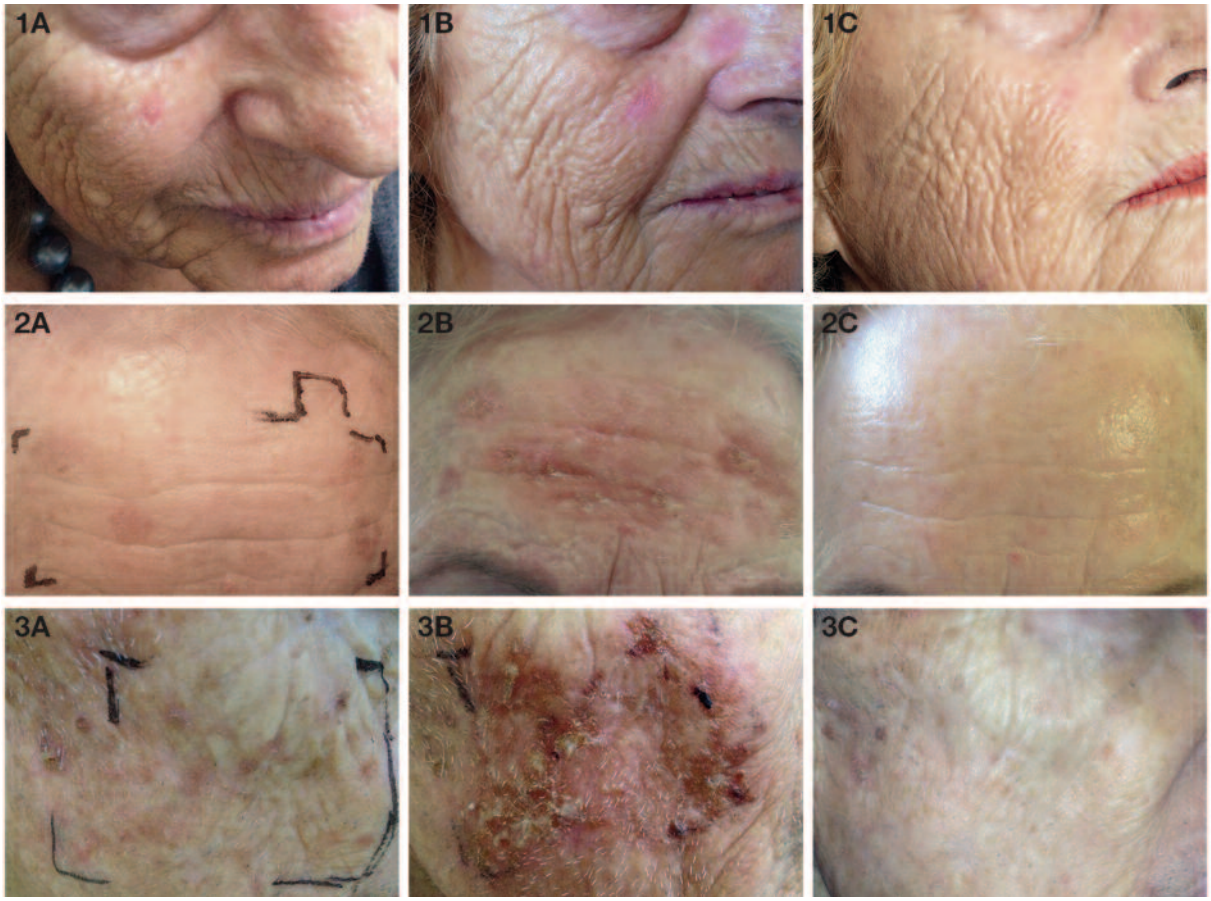

Figure 1. Clinical pictures of 3 different patients at baseline, during LRS and after treatment with ingenol mebutate 0.015%, for 3 consecutive days. **Patient n. 1** ***A,*** A Caucasian female showing multiple non hypertrophic actinic keratosis on the nose and left cheek at baseline. ***B,*** Same patient after 4 days of application of ingenol mebutate gel 0.015 showing low-grade inflammation, just slight erythema and oedema without blistering, erosion and crusts. ***C,*** Same patient, after 60 days of treatment showed a complete resolution with great cosmetic results, healthy skin and complete AKs clearance. **Patient n. 2** ***A,*** Caucasian female, with multiple non hypertrophic actinic keratosis on her forehead at baseline. ***B,*** Same patient after 4 days of ingenol mebutate 0.015% application, we observed a mild local skin reaction with oedema, erythema, erosion and crusts. ***C,*** Same patient, with a complete resolutions of LRS and healthy skin, without scars and complete AKs clearance after 60 days. **Patient n. 3** ***A,*** A Caucasian male with multiple non hypertrophic actinic keratosis on the left check at baseline ***B,*** Same patient, after 4 days of ingenol mebutate 0.015% application showing a severe local skin reaction, with severe erythema, oedema, blistering, sterile pustules also outside application area and erosions. ***C,*** Same patient, after 60 days of application, a complete resolution with evident healthy skin, with no scars and a complete AKs cleareance.

focused on the management of the whole actinically damaged field[6]. Recently approved in the italian market, ingenol mebutate, in two formulations (0.05% trunk and extremities 0.015% face and scalp), for the treatment of multiple non-hypertrophic AKs, has giving interesting results[7]. We evaluated its efficacy with Antera 3D (Miravex, Limited, Dublin, Ireland)[8].

Patients and Methods

A retrospective data analysis was performed in the AKs unit of our dermatological department. All patients signed consent forms and were informed about the treatments procedures and local skin reactions (LRS). We included in the analysis all patients with multiple non-hypertrophic actinic keratosis to whom it was prescribed ingenol-mebutate gel, 0.015% for lesions in the scalp (for 3 consecutive days) and 0.05% for lesions in the trunk and/or extremities (for 2 consecutive days). The aim of the current report was not to evaluate the clinic-pathological predictors for a better response to treatment, while its efficacy using a novel device the *in vivo* optical skin imaging (Antera 3D, Miravex, Limited, Dublin, Ireland) which

allows the view of skin in 2 or 3 dimensions as well as multi-spectral analysis. Patients were collected according to sex, age (≤ 60 or ≥61 years), reduction in number of cutaneous lesions, satisfaction (reduction of AK and an aesthetic performance), vascularization and inflammation severity. The image acquisition was performed at baseline, during LRS and after the treatment and retrospectively evaluated, according to treated lesion's vascularization severity and relative inflammation. This parameter was evaluated considering the concentration of hemoglobin (directly proportional to the grade of vascularization) with Antera 3D.

Statistical Analysis

Assuming that the effects of the variables are constant over time, an Odds Ratio (OR) was performed to evaluate the number of clinical responses and morphological evaluations at the imaging device. A p value < 0.05 was considered statistically significant.

Results

A total of 65 patients treated with ingenol-mebutate gel for AKs were enrolled in current report. Thirty-five case series of patients were male (54%) and 58 were ≥ 61 years old (89%). A reduction of the lesions (as a reduction in the number of lesions ≥ 51% of the total number of lesions), after a follow-up of 60 days, was observed in 100% of patients. At the same time the patient's satisfaction increased directly with the disappearance of Aks (97% after 60 days) (Figure 1). Starting from these results we decided to evaluate AK's vascularization before and after treatment, as well as the inflammation degree. A measurement of hemoglobin was used to identify tiny blood vessels and follows the treatment efficacy. A reduction of median hemoglobin levels was observed in all patients analyzed. Performing Odds-Ratio, a statistical significance was reached, showing a $p < 0.001$ (OR: 201). At the same time we observed an inflammation reduction after the treatment, showing an OR of 0.4 with a p value < 0.05 (Figures 2, 3). In conclusion, vascularization of the treated areas showed a reduction after a follow-up of 60 days, with a relative clinical reduction of inflammation, associated also with an aesthetic performance, as reported by the high value of patient's satisfaction.

Discussion

AKs are considered a worldwide problem with continuously increasing incidence, considered as an early step in the continuum of transformation from normal skin to invasive SCC[9]. The new treatment approach is focused on possible recurrence of existing lesions versus emergence of new AK lesions in a similar area (actinic field). In this regards, ingenol mebutate gel, applied as field therapy, was retretrospectively considered for the treatment of multiple non-hypertrophic AKs. The results of our analysis showed that ingenol mebutate gel was effective when treating head or body actinic keratoses, producing clinically relevant clearance and reduction in the number of lesions in the selected field due to its dual mechanism of action, that combines rapid direct cell death with a neutrophil-mediated immune response[10]. According to literature, LRs were experienced in the majority of our treated patients, although they resolved after 15 days, without scars or skin colour change (often seen with criosurgery), without evidence of skin sensitization, photoirritation, or photoallergy, they did not need any additional treatment and did not influence patient's adherence to treatment, since we explained what could happen during the first visit[11]. As observed in clinical pictures, a complete resolution was achieved with an improvement in skin quality (after 60 days), in fact patients were satisfied about clinical efficacy and aesthetic outcome, they had the feeling of visible healthy skin. We also evaluated its efficacy using *in vivo* optical skin imaging, which showed tridimensional appearance of the skin, vascular pattern, melanogenic distribution and wrinkles, focusing our interest on the vascular aspect[12]. As known, metastatic spread, of cancer cells depends on an adequate supply of oxygen and nutrients and the removal of waste products. In this regard, tumor growth and metastasis depend on neo-angiogenesis and lymphangiogenesis, triggered by chemical signals from tumor cells in a phase of rapid growth[13]. Pathological angiogenesis occurs in skin cancers and is a crucial pathway in tumor biology in general. In fact, certain stimuli promote a pro-angiogenic microenviroment such as acute ultraviolet damage, which causes dramatic changes in growth factor cytokines in normal skin[14]. Transformed lesions are capable of growth up

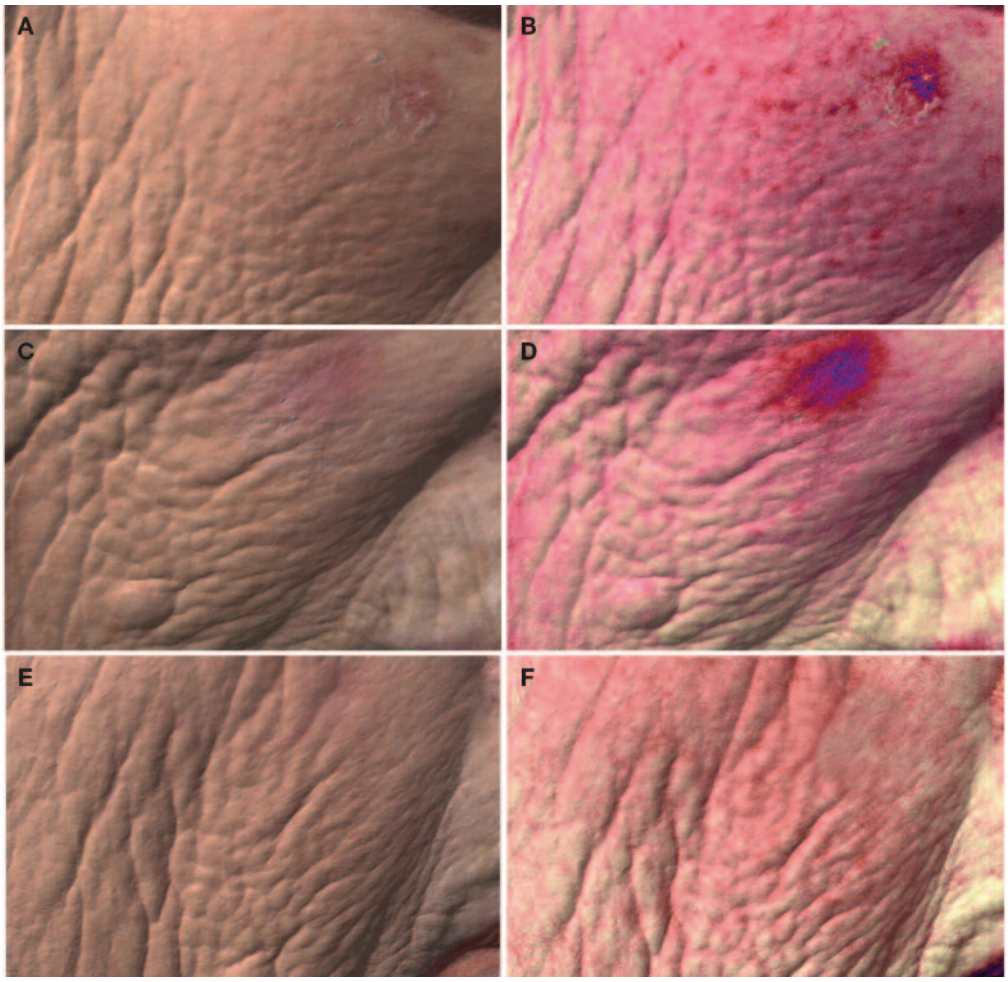

Figure 2. Images of the same patient have been acquired with Antera® (Miravex). ***A,*** Clinical tridimensional image acquired with Antera® (Miravex) showing multiple actinic keratosis over the zygomatic and nasal region at baseline. ***B,*** The hemoglobin analysis using Antera® (Miravex), showed a pseudo-crateriform shape of the actinic keratosis with an high vascular component at baseline. After 4 days of application of Ingenol mebutate gel 0.015% a mild inflammation was observed in the treated area, as showed by figures ***C*** (tridimensional) and ***D*** (hemoglobin distribution). ***E,*** Tridimensional image showed a complete lesions resolution after a follow up of 60 days, ***F,*** An important decrease of mean hemoglobin values (directly related to a reduction of vascularization) was observed.

to 2 mm in diameter before their metabolic demand exceed the available blood supply leading the switch to the angiogenic phenotype. In fact, compared to physiological skin, AKs show an increment of vascularization (pathological neo-angiogenesis) and exhibit capillary densities greater than surrounding normal tissue, which becomes more severe and evident in invasive tumoral lesions. As showed in the current study, the use of ingenol-mebutate gel has reduced the median levels of hemoglobin (directly related to the vascularization) showed by Antera ($p < 0.001$), supposing a possible reduction of the risk of tumoral progression. Contrasting this process will be useful in the prevention and early intervention in skin cancers. In this view, our study, focused on vascular pattern, demonstrated the reduction of hemoglobin content in Aks, after ingenol application, allowing a better therapeutical approach to every single lesion. A reduction of median hemoglobin levels was observed after 60 days, showing a reduction of AK's vascularization. However, further studies are needed to corroborate this hypothesis. This simple camera could be considered an useful tool in the everyday dermatological practice to evaluate treatment follow-up.

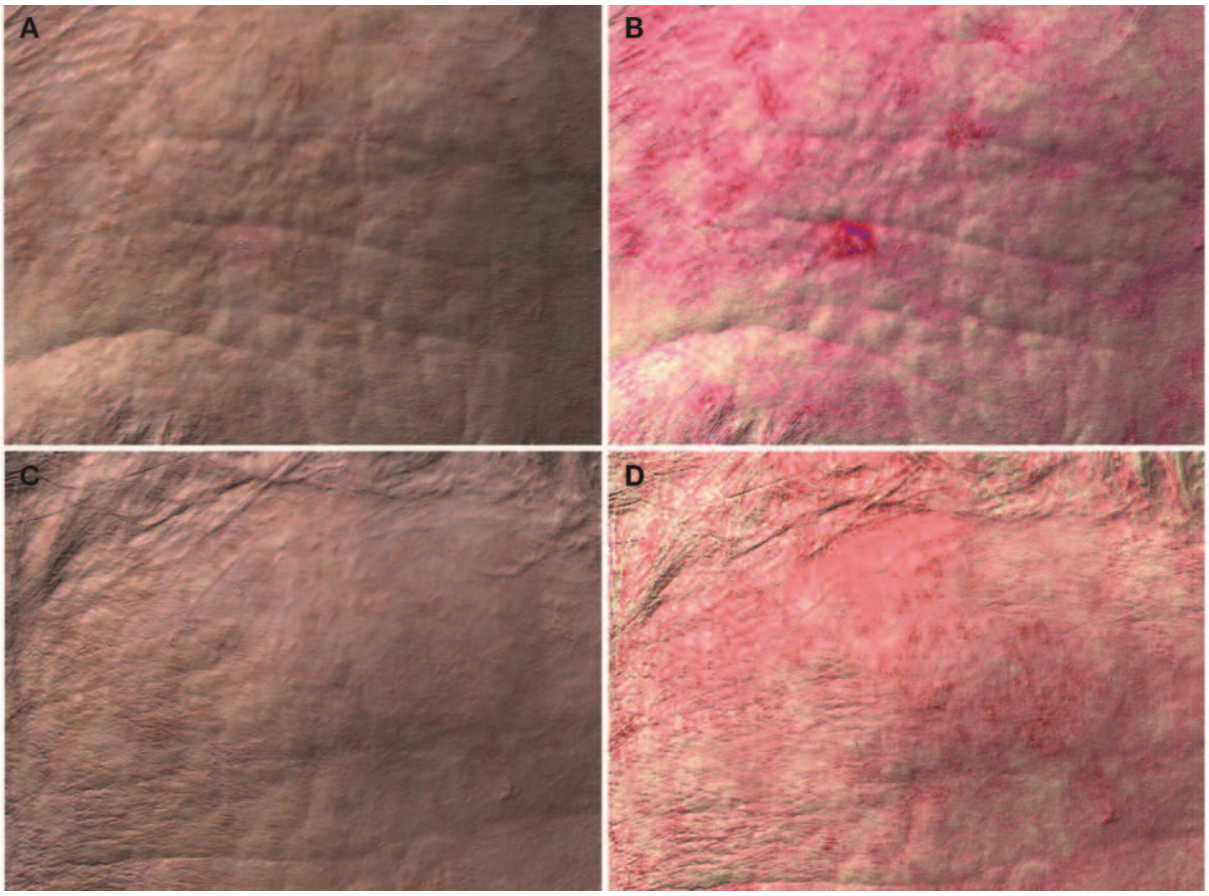

Figure 3. Images of the same patient have been acquired with Antera® (Miravex). ***A,*** Clinical tridimensional image showed multiple non hypertrofic actinic keratosis in a photodamaged skin at baseline (A tridimensional view-B hemoglobin distribution). ***B,*** Showed multiple "red spots", corresponding to high levels of hemoglobin and high vascularization at baseline. ***C,*** 60 days after treatment, disappearance of actinic keratosis was observed in tridimensional image and ***D***. As showed also by the reduction of intensity of the "red spots" (hemoglobin and vascular components). A reduction of the lesions (as a reduction in the number of lesions ≥ 51% of the total number of lesions), after a follow-up of 60 days, was observed in 100% of patients in follow-up.

Conclusions

Ingenol mebutate can be considered an effective, innovative and cost effective treatment accepted by patients especially for its short duration (just two or three consecutive days of application), which is changing italian style to treat AKs in the near future[15]. Ingenol mebutate, can be used the whole year around as first line treatment.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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