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Ingenol Mebutate: An Emerging Therapy in the Treatment of Actinic Keratoses

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Abstract Ingenol mebutate gel, derived from the sap of the *Euphorbia peplus* plant, recently has been investigated as a potential topical treatment for actinic keratoses. The medication is proposed to have a dual mechanism of action: rapid lesion necrosis followed by lesion-specific, neutrophil-mediated, antibody-dependent cellular cytotoxicity. A literature review of the data from phase II and III clinical trials reveals that ingenol mebutate gel is an efficacious and safe therapy for actinic keratoses when used on areas of skin up to 25 cm^2. Further research is needed to compare directly ingenol mebutate gel to other treatments available for actinic keratoses, as well as to establish safety and efficacy for application on larger areas of skin.

Keywords Ingenol mebutate · Actinic keratosis · *Euphorbia peplus*

Introduction

Actinic keratoses are premalignant lesions commonly encountered in dermatology, with risk factors that include fair skin types, age, and a history of chronic sun exposure [1]. Cryotherapy is the most widely utilized treatment, but it is associated with the risk of scarring. Topical therapies, such as 5-fluorouracil or imiquimod, are disadvantageous for other reasons, including the longer duration of treatment and the risk of localized skin reactions with prolonged application, both of which may negatively impact patient adherence to treatment [2, 3••].

Recently, the medical community has focused its attention on a new treatment for actinic keratoses called ingenol mebutate. This medication is derived from the sap of the *Euphorbia peplus* plant, also known as petty spurge, radium weed, or milkweed. The sap is a white, sticky irritant that has long been used in traditional medicine for treatment of warts, corns, and nonmelanoma skin cancers [4]. An Australian survey from 1986 regarding the use of home remedies for skin cancers and actinic keratoses described support among respondents regarding the effectiveness of the sap of *Euphorbia peplus* [5]. Given the long history of use in traditional medicine, as well as the absence of documented adverse effects, recent clinical trials have been designed to further investigate the effectiveness and safety profile of ingenol mebutate.

In January 2012, ingenol mebutate gel attained FDA approval for the treatment of actinic keratoses. For areas on the face and scalp, ingenol mebutate 0.015 % gel is approved for application once daily for 3 consecutive days for an area of skin up to 25 cm^2. For areas on the trunk and extremities, ingenol mebutate 0.05 % gel is approved for application once daily for 2 consecutive days, also for an area up to 25 cm^2 [6]. The medication is a clear colorless gel that is dispensed in cartons containing either three single-use, 0.25-g tubes of 0.015 % ingenol mebutate gel or two single-use, 0.25-g tubes of 0.05 % gel.

Mechanism of Action

Whereas the precise mechanism of ingenol mebutate is unknown, this diterpene ester (Fig. 1) is proposed to have

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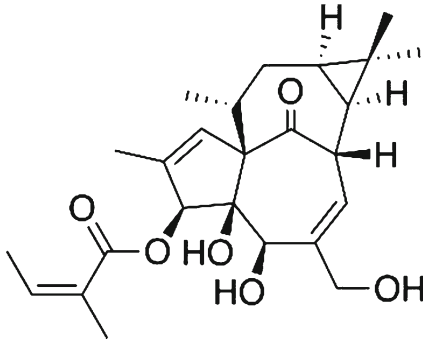

Fig. 1 Structure of ingenol mebutate (ingenol 3-angelate), an ingenol ester

a dual mechanism of action: rapid lesion necrosis followed by lesion-specific neutrophil-mediated, antibody-dependent cellular cytotoxicity (ADCC) [7••]. It is worth noting that there have been no studies of the mechanism of action of ingenol mebutate in humans, and there are no animal models for actinic keratoses, so the above hypothesis is derived from other tumor models in animals, including the B16 mouse melanoma cell line and the KL2 mouse squamous cell carcinoma cell line [8, 9].

The first aspect of the proposed mechanism of action, rapid lesion necrosis, is based on observations of changes in both the mitochondrial membranes and plasma membranes of tumor cells soon after exposure to ingenol mebutate [9]. It is hypothesized that ingenol mebutate is taken up by cells via endocytotic vesicles, which in turn release Ca^{+2} into the cytoplasm [10, 11]. With the rise in intracellular Ca^{+2}, mitochondrial membrane potential is disrupted, leading to loss of adenosine triphosphate production [7••]. Electron microscopy studies have documented swelling of the mitochondria, disruption of other organelles, and loss of plasma membrane integrity within hours of exposure to ingenol mebutate [7••, 9]. All of these findings lend support to the idea of necrosis, rather than apoptosis, as the mechanism of cell death. The necrosis is thought to occur quite rapidly, within 1 hour of application of ingenol mebutate [7••, 9].

Neutrophil-mediated ADCC is the second aspect of the proposed mechanism. As the targeted cells undergo necrosis, the release of cytokines attracts an inflammatory infiltrate that is composed predominately of neutrophils [8]. The abundance of neutrophils at the site of treatment approximately 24 hours after application coincides with clinical evidence of inflammation, which slowly resolves over 5-10 days [7••]. As major players in the innate immune response, the main role of neutrophils is phagocytosis. However, neutrophils also are capable of recognizing cells coated with antibody via their Fc receptors, which can trigger killing of target cells through release of reactive oxygen species, a process known as ADCC. Ingenol mebutate, through unclear mechanisms, also increases production of antibodies by B cells, including tumor-specific antibodies, which likely enhances the process of neutrophil-mediated ADCC [7••, 8]. Interestingly, in neutrophil-depleted mice treated with ingenol mebutate, tumor ablation initially occurred, likely due to lesion-specific necrosis, but 83 % of the mice had tumor regrowth in a period of approximately 3 months [8]. Similar relapses were noted in B cell–deficient mice with severe combined immunodeficiency [8]. This suggests that neutrophils play an important role in preventing recurrence of tumors following application of ingenol mebutate. Whether this will translate to a lower rate of recurrence of actinic keratoses in human patients treated with ingenol mebutate remains to be seen.

Clinical Data

Topical ingenol mebutate gel has been the subject of several recent clinical trials as a potential therapy for actinic keratoses. A review of the literature, including phase II and III clinical trials, suggests that ingenol mebutate is an efficacious and safe treatment for actinic keratosis.

Efficacy

Phase II Data

In a multicenter, randomized, double-blinded, vehicle-controlled, phase IIa study, Siller et al. evaluated the safety and efficacy of ingenol mebutate gel at varying concentrations [12•]. A total of 58 Caucasian patients, ranging in age from 44 to 86 years, with at least five clinically diagnosed actinic keratoses on the arms, shoulders, chest, face, or scalp were included. The gel (at concentrations of 0.0025 %, 0.01 %, 0.05 %, or placebo) was applied directly to five preselected actinic keratoses on day 1 and day 2 (arm A) or day 1 and day 8 (arm B).

Efficacy, determined by clinical improvement, was evaluated on day 85. There were no statistically significant differences in the efficacy data from arm A and arm B; thus, the data were combined. Response did vary depending on the concentration of ingenol mebutate gel. Actinic keratoses treated with 0.05 % gel had the highest rates of clinical improvement (53/75 lesions completely cleared, 71 %). A comparison between the vehicle gel placebo group and the treatment groups resulted in a statistically significant ($P<$ 0.0001) difference in rates of clinical clearance [12•].

Following early phase I and phase 2a studies supporting the favorable safety profile and appropriate dosing regimen of ingenol mebutate, Anderson et al. conducted a phase 2b study across 22 U.S. centers to further evaluate this promising drug [13]. A total of 222 Caucasian patients, age 43-85 years, with 4 to 8 clinically typical actinic keratoses in a 25 cm^2 contiguous area on the arm, shoulder, chest, back, or

scalp were enrolled in a double-blind, double-dummy, vehicle-controlled trial. Patients were randomized to treatment with ingenol mebutate 0.025 % daily for 3 days, ingenol mebutate 0.05 % daily for 3 days, ingenol mebutate 0.05 % for 2 days, or treatment with vehicle gel.

Partial clearance rates (the proportion of patients at day 57 with at least a 75 % reduction in the number of clinically identifiable actinic keratoses) and complete clearance rates (the proportion of patients at day 57 with no clinically visible actinic keratoses in the treatment field) were the primary and secondary end points, respectively. All three treatment arms showed higher clearance rates than the vehicle arm, with a clear dose-response relationship. Partial clearance was seen in 56 % of patients treated with 0.025 % for 3 days, 61.8 % of patients treated with 0.05 % for 2 days, 75.4 % of patients treated with 0.05 % for 3 days, and 21.7 % of the vehicle treatment group. Complete clearance was noted in 40 % of patients treated with 0.025 % for 3 days, 43.6 % of patients treated with 0.05 % for 2 days, 54.4 % of patients treated with 0.05 % for 3 days, and 11.7 % of the vehicle treatment group. Patients treated with ingenol mebutate had a 75-100 % median percentage reduction in baseline actinic keratosis lesions compared with 0 % for vehicle gel ($P<0.0001$ versus vehicle) [13].

Phase III Data

Based on encouraging data from phase II studies evaluating the safety and efficacy of ingenol mebutate gel [12•, 13], phase III trials were initiated in 2008. Lebwohl et al. reported a phase III trial that included four multicenter, randomized, parallel-group, double-blind, vehicle-controlled studies evaluating both the efficacy and safety of ingenol mebutate gel [3••]. Caucasian (mostly Fitzpatrick skin types I and II) patients age 18 years and older (mean age 61.5 years), with four to eight discrete actinic keratoses were randomly assigned to self apply ingenol mebutate gel or placebo (vehicle gel) to a 25 cm^2 contiguous field. Two of the four trials evaluated patients with actinic keratoses involving the face and scalp with application of ingenol mebutate gel 0.015 % for 3 consecutive days (n=547). The remaining two trials evaluated patients with actinic keratoses on the trunk and extremities with application of ingenol mebutate gel 0.05 % for 2 consecutive days (n=458).

Efficacy was based on the evaluation of patients at baseline and on day 57. Complete clearance of all clinically visible actinic keratoses within the area of treatment was the primary end point. A reduction of at least 75 % of the amount of clinically visible actinic keratoses (partial clearance) on day 57 was defined as the secondary end point.

Complete and partial clearance of actinic keratoses of the face and scalp were significantly higher when ingenol mebutate gel was compared to vehicle gel (placebo): 42 % versus 3.7 % for complete clearance ($P<0.001$) and 63.9 % versus 7.4 % for partial clearance ($P<0.001$), respectively [3••]. Pooled data from the two face and scalp trials showed complete clearance rates were higher for the face (47.3 %) than for the scalp (22.8 %) [14].

In the trunk and extremity trials, complete and partial clearance of actinic keratoses were significantly higher in the ingenol mebutate group versus the vehicle gel group: 34.1 % versus 4.7 % for complete clearance ($P<0.001$) and 49.1 % versus 6.9 % for partial clearance ($P<0.001$) respectively [3••]. Pooled data from the two trunk and extremity trials showed lower complete clearance rates in the ingenol mebutate group for the back of the hand (18.5 %) versus the arm (34.5 %) and the chest, leg, back, and shoulder (60 %) [15].

Long-term follow-up was addressed by this phase III trial in an observational arm involving three of the four study populations, which followed those patients with complete clearance at day 57 for an additional 12 months. At a 12-month follow-up of 108 patients who used ingenol mebutate on the face and scalp with complete clearance at day 57, 87.2 % of the resolved actinic keratoses remained clear. One or more new actinic keratoses were observed in 53.9 % of these patients. In 38 patients who received ingenol mebutate to the trunk and extremities with complete clearance by day 57, 85.1 % of the resolved actinic keratoses remained clear. One or more new actinic keratoses were observed in 50 % of the patients [3••].

Schmieder et al. reported a multicenter, open-label, phase 3b study evaluating the safety of ingenol mebutate gel [16]. As a secondary objective, the efficacy of ingenol mebutate gel for the treatment of actinic keratoses on the trunk or extremities was evaluated. The study (N=102) examined the clearance of actinic keratoses with daily self-applied ingenol mebutate gel 0.05 % for 2 consecutive days. Patients were at least 18 years of age, with 4-8 clinically discrete actinic keratoses within a 25-cm^2 contiguous field involving the trunk or extremities.

Efficacy was based on clearance and the total percentage of reduction in lesions. At day 57, complete clearance was achieved in 39.2 %, and partial clearance (defined as ≥75 % clearance) was achieved in 54.9 % of overall patients. The median number of actinic keratoses within the treatment area from baseline to day 57 was reduced by 75 % [16]. Lower rates of clearance were seen in actinic keratoses treated on the dorsal hands, a finding which underscores the difficulty in treating actinic keratoses on the dorsal hands.

Nonmelanoma Skin Cancer

In addition to its efficacy as a treatment option for actinic keratoses, ingenol mebutate has been shown to be effective

for the topical treatment of squamous cell carcinoma in situ and squamous cell carcinoma (SCC), both often considered on a continuum with actinic keratoses.

Ramsay et al. conducted a phase I/II clinical study to determine the effectiveness of *Euphorbia peplus* sap for the topical treatment of squamous cell carcinomas and intraepidermal carcinomas (IEC) (basal cell carcinomas also were included in the study but will not be discussed here) [4]. Twenty patients, at least age 18 years, with biopsy-proven IEC (N=16) or SCC (N=4) were enrolled in the study. All patients had either failed previous treatments and refused surgery or were considered poor surgical candidates. The IEC/SCC lesions were treated once daily for 3 consecutive days with 100-300 μL of *Euphorbia peplus* sap. There was a complete response at last follow-up (mean 15 months, range 2-31 months) in IEC and SCC of 75 % and 50 %, respectively.

Safety

In the phase III trial by Lebwohl et al., ingenol mebutate was well tolerated overall. Safety was evaluated the day following the last topical application (day 4 for scalp and face and day 3 for trunk and extremities) and days 8, 15, 29, and 57 [3••]. As expected, application site adverse reactions were the most commonly reported conditions in all four trials. The trial utilized the local-skin-response scale that graded the following six parameters on a 0 (no reaction) to 4 (severe reaction) scale: erythema, flaking or scaling, crusting, swelling, vesiculation or pustulation, and erosion or ulceration. A composite score was then derived (maximal score was 24). For the face and scalp, the maximal composite score was 9.1±4.1 versus 1.8±1.6 for the ingenol and placebo groups, respectively. The composite scores for the trunk and extremity groups were 6.8±3.5 versus 1.6±1.5, respectively. Local skin reactions in patients treated with ingenol mebutate on the face and scalp versus placebo were erythema (69.7 % vs. 2.2 %), pain (13.9 % vs. 0.4 %), pruritus (8 % vs. 1.1 %), and irritation (1.8 % vs. 0 %), respectively. In this cohort, the composite score peaked at day 4 and declined to near baseline by day 15. In the patients treated with ingenol mebutate applied to the trunk and extremities versus placebo, pain (2.2 % vs. 0 %), pruritus (8.4 % vs. 0 %), and irritation (3.6 % vs. 0.4 %) were noted, respectively. The composite score peaked at day 3 for 55.1 %, day 8 for 32.4 %, and day 15 for 8.4 %. Local-skin-response scores were near baseline at day 29 in this group. There were no reported serious adverse events related to the medication. Though not quantified, the authors state there was minimal scarring or change in pigmentation in all four studies [3••].

Conclusions

Actinic keratoses are premalignant lesions that are common in light-skinned populations [17]. There are numerous lesion-specific and field therapies, including cryotherapy, topical imiquimod, topical 5-fluorouracil, topical diclofenac, photodynamic therapy, medium and deep chemical peeling, dermabrasion, and ablative laser resurfacing. The therapy chosen depends on patient characteristics, extent of disease, and tolerability. Ingenol mebutate, the active compound in the sap of *Euphorbia peplus*, is a new topical therapeutic that has shown efficacy in the treatment of actinic keratoses [3••].

There is a proposed dual mechanism of action involving rapid lesion necrosis followed by lesion-specific, neutrophil-mediated, antibody-dependent cellular cytotoxity [7••]. However, the precise mechanism of action remains incompletely understood and is the source of ongoing investigations.

The efficacy of ingenol mebutate is comparable to other topical treatments. Imiquimod has complete clearance rates ranging from 30.6 % (2.5 % for 2 weeks on, 2 weeks off, 2 weeks on) [18] to 35.6 % (3.75 % for 2 weeks on, 2 weeks off, 2 weeks on) [18] to 45.1 % (5 % two times a week for 16 weeks) [19]. One study of 5-fluorouracil daily for 4 weeks showed a complete clearance rate of 47.5 % [20]. The primary benefit of ingenol mebutate is its shorter duration of treatment, which is more convenient for patients and presumably results in higher compliance rates. Further research is needed to compare directly the efficacy of ingenol mebutate gel to other currently available treatment regimens for actinic keratoses.

Ingenol mebutate gel was well-tolerated overall. The primary side effects were local skin reactions that were of short duration. The local-skin-response scores returned to near baseline levels in 15 days for those patients treated on the face and scalp and in 29 days for those patients treated on the trunk and extremities [3••].

The high clearance rates along with a favorable safety profile make ingenol mebutate an important addition to the current therapeutic options for actinic keratoses. Limitations of the medication include lower response rates when applied to the dorsal hands and the scalp compared with other areas of the body. Further research is needed to investigate the application of ingenol mebutate gel on larger areas of skin, because the medication is currently approved for application on areas up to only 25 cm^2, as well as to compare directly the safety, efficacy, and tolerability of the medication to other treatments for actinic keratoses.

Conflict of Interest Stephanie Jacks declares that she has no conflict of interest.

Kasie Kudrewicz declares that she has no conflict of interest.

David R. Carr declares that he has no conflict of interest.

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- • Of importance
- •• Of outstanding importance

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