

Clinical efficacy assessment in photodamaged skin of 0.5% and 1.0% idebenone

Summary Idebenone is an antioxidant lower molecular weight analogue of coenzyme Q10. Previously, idebenone was shown to be a very effective antioxidant in its ability to protect against cell damage from oxidative stress in a variety of biochemical, cell biological, and *in vivo* methods, including its ability to suppress sunburn cell (SBC) formation in living skin. However, no clinical studies have been previously conducted to establish the efficacy of idebenone in a topical skincare formulation for the treatment of photodamaged skin. In this nonvehicle control study, 0.5% and 1.0% idebenone commercial formulations were evaluated in a clinical trial for topical safety and efficacy in photodamaged skin. Forty-one female subjects, aged 30 –65, with moderate photodamaged skin were randomized to use a blind labelled (either 0.5% or 1.0% idebenone in otherwise identical lotion bases) skincare preparation twice daily for six weeks. Blinded expert grader assessments for skin roughness/dryness, fine lines/wrinkles, and global improvement in photodamage were performed at baseline, three weeks and six weeks. Electrical conductance readings for skin surface hydration and 35 mm digital photography were made at baseline after six weeks. Punch biopsies were taken from randomly selected subjects, baseline and after six weeks, and stained for certain antibodies (interleukin IL-6, interleukin IL-1b, matrixmetalloproteinase MMP-1, collagen I) using immunofluorescence microscopy. After six weeks' use of the 1.0% idebenone formula, a 26% reduction in skin roughness/dryness was observed, a 37% increase in skin hydration, a 29% reduction in fine lines/wrinkles, and a 33% improvement in overall global assessment of photodamaged skin. For the 0.5% idebenone formulation, a 23% reduction in skin roughness/dryness was observed, a 37% increase in skin hydration, a 27% reduction in fine lines/wrinkles, and a 30% improvement in overall global assessment of photodamaged skin. The immunofluorescence staining revealed a decrease in IL-1b, IL-6, and MMP-1 and an increase in collagen I for both concentrations.

Keywords: antioxidant, idebenone, photodamage

Introduction

Antioxidants have become popular anti-aging ingredients in topical skincare products. Antioxidants have been shown to be photoprotective, anti-inflammatory, able to reduce UVR-induced immunosuppression, and protective against free radical-mediated cellular damage. Typically these ingredients have been referred to as agents that

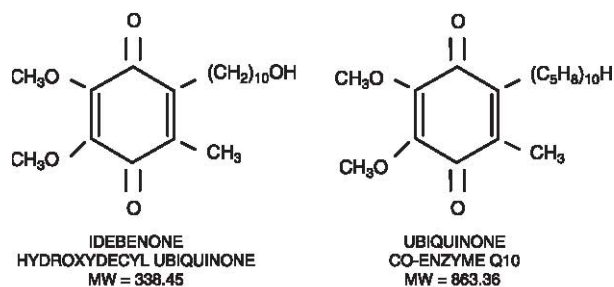


Figure 1 Structure and molecular weight of idebenone vs. Coenzyme Q10.

are protective in nature (i.e., excellent for treating the cause of aging) but offer little benefit from an efficacy standpoint in their ability to reverse the signs of skin aging (i.e., treating the effects of skin aging). Although there has been significant research relating to antioxidant protective benefits, there has been very little published clinical research that would demonstrate efficacy in the treatment of aging skin or photodamaged skin. Some data have been introduced for vitamin C and coenzyme Q10 in this regard, but generally, clinical data to support antioxidant efficacy in the treatment of photodamaged skin are lacking.²⁻⁵

In this clinical research, we assessed the safety and efficacy of topical skincare preparations containing 1.0% and 0.5% idebenone, a novel new antioxidant for skin-care, in the treatment of photodamaged skin. Previously idebenone was compared against commonly known popular antioxidants in skincare products (vitamin C, vitamin E, alpha lipoic acid, kinetin, and coenzyme Q10) and shown to be very effective in its ability to protect against damage as a result of oxidative stress in a variety of biochemical, cell biological, and *in vivo* methods. In this research, idebenone was shown to be the most effective antioxidant in overall global assessment to prevent oxidative stress and received the highest environmental protection factor (EPF rating) for protection from oxidative stress of the antioxidants tested.⁶

The chemical structure of idebenone is very similar to coenzyme Q10 (see Fig. 1). Idebenone is a lower molecular weight (approximately 60% smaller) analogue to coenzyme Q10. In theory this should aid in the penetration of this molecule, compared to that of Coenzyme Q10 in topical applications. Coenzyme Q10 is a very important electron transfer agent of the respiratory chain in the mitochondria of our cells, the source of metabolic energy production. However, unlike Coenzyme Q10, idebenone protects against radical formation and cell damage under hypoxic (low oxygen) cellular stress situations. Under these same conditions, Coenzyme Q10

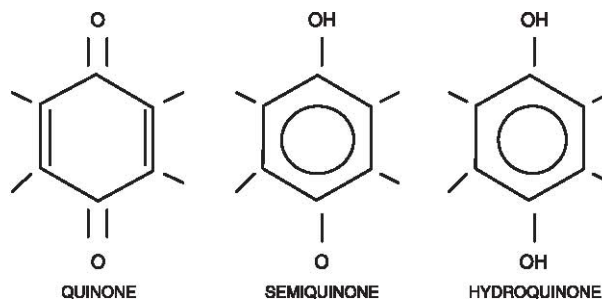


Figure 2 Core structure of idebenone demonstrating quinone, semiquinone, and hydroquinone redox forms.

is known to auto-oxidize, becoming a very potent pro-oxidant, leading to the production of hydrogen peroxide, superoxide, and hydroxy radicals in massive numbers.⁷ In addition, it has a structure similar to hydroquinone (see Fig. 2), occurring in all various chemical forms (quinone, semiquinone, and hydroquinone) depending on the cellular oxidation–reduction (redox) situation. Therefore, one might expect it to have a similar inhibiting action to increased melanin production (hyperpigmentation) by the melanocytes. Thus, idebenone, both a powerful antioxidant lower molecular weight analogue to coenzyme Q10 and a mimic molecule of hydroquinone, might be expected to deliver clinically visible results in the treatment of photodamaged skin.

This study was designed to elicit clinical safety and efficacy for a topical preparation containing idebenone at two different dose concentrations (0.5% and 1.0% w/w) and investigate for possible mechanisms of action. The concentrations chosen were selected based on results obtained from previous studies conducted via biochemical, cell biological, and *in vivo* methods. In particular, a sunburn cell (SBC) dose–response study revealed that idebenone demonstrated significant efficacy at 0.5% (38% reduction in SBCs) and was even more effective at a 1.0% concentration (44% reduction in SBCs). In general, it is known, that there are several major matrix degradation pathways that lead to premature aging of the skin (see Fig. 3).⁸ Both the cJun/cFos genes (AP1 transcription factor) pathways that affect MMP/collagen synthesis and the nuclear factor kappa beta transcription factor (NFκB)/interleukin pathways which affect inflammatory response have as a common denominator their initiation by free radical–mediated oxidative stress. Inhibition of either pathway may improve the overall appearance of photoaged skin. Specifically, in randomly selected subjects participating in the clinical trial, skin biopsies were taken and assessed for the presence of certain biomarkers that are key components of both degradation pathways (IL-6, IL-1b, MMP-1, and collagen I) utilizing immunofluorescence staining techniques.⁹ A decrease in MMP activity

Table 1 Inclusion/exclusion criteria for study.

Inclusion Criteria:	<ol style="list-style-type: none"> 1. Subjects with moderate photoaging must be diagnosed by the investigator. <ol style="list-style-type: none"> 1. Subjects must be female and preferably above 30 years of age with no known medical conditions that, in the investigator's opinion, may interfere with study participation. 2. Subjects must discontinue all current photoaging products. 3. Subjects must provide written informed consent and photography consent.
Exclusion Criteria:	<ol style="list-style-type: none"> 1. Any dermatological disorder or personal appearance issue which, in the investigator's opinion, may interfere with the accurate evaluation of the subject's face. <ol style="list-style-type: none"> 1. Subjects who have demonstrated a previous hypersensitivity reaction to any ingredients in the study products. 2. Concurrent therapy with any medication either topical or oral that might interfere with the study. 3. Subjects who have undergone any surgical treatment to the tissues of the face. 4. Subjects who are not willing to discontinue all anti-aging prescription or OTC cosmeceutical preparations to the face. 5. Subjects who have participated in another clinical trial or have taken an experimental drug within the past 30 days. 6. Subjects who are pregnant, breast-feeding, or planning a pregnancy. 7. Subjects who are unwilling or unable to comply with the requirements of the protocol.

Figure 3 Pathways of aging skin.

may result in a net increase in collagen and a decrease in inflammation, particularly IL-6 may also reduce MMP and thus produce changes in the dermal matrix that may provide the mechanism of action for the clinical results seen in the study.

Methods, materials, and study design

Fifty female subjects with moderate photoaging (dyschromic facial skin with fine lines and wrinkles) between the ages of 30 and 65 were enrolled in the study and randomly divided into two equal groups of 25 subjects each. Subjects were given a commercial water-oil-water (w/o/w) emulsion lotion containing either 1.0% or 0.5% idebenone w/w in blinded containers. Of the 25 subjects receiving the 1.0% formulation, 21 completed the study; of the 25 subjects receiving the 0.5% formulation, 20 completed the study. All study dropouts were for personal reasons and none were related to the efficacy, safety, or adverse events from the products. A lotion base, as opposed to a gel or cream was chosen because this is, by overwhelming preference, the most popular base for the general consumer population. The subjects were instructed to apply the product to the entire facial area twice daily (b.i.d.) morning and evening for a period of six weeks. Twice daily application was chosen to maximize efficacy benefit and at the same time to evaluate maximum use to reveal any potential skin sensitivity problems or adverse reactions (in order to better assess the safety of the idebenone formulations at each

concentration). A study duration time of six weeks was chosen because marketing research has shown that consumers are not likely to continue use of an anti-aging cosmetic product if they cannot see visible results within a relatively short period of time. Six weeks was also chosen to enable comparison to other short-term studies typical for similar cosmetic anti-aging products. All subjects received active product (either the 0.5% or 1.0% formulation) and no placebo was employed in the study. This study design was chosen to maximize dose comparison safety and efficacy, minimize overall cost, and eliminate crossover mix-up or contamination that could potentially occur in a split face active/placebo or vehicle control design as well as to provide specific information concerning safety and efficacy for a finished product formulation intended for market release. [Note: These study results highlight the safety and efficacy of a final product formulation rather than the specific ingredient idebenone. It should be noted that no other known anti-aging ingredients were employed in the tested formulation, and therefore, any increase in efficacy over the results one would expect from an ordinary water-oil-water emulsion should reasonably be attributed to the incorporation of idebenone.] Specific subject inclusion and exclusion criteria are given in Table 1. Subjects were allowed to continue their normal facial cleansing routine and makeup products and instructed to apply the test product to clean, dry facial skin. Subjects were also given an SPF 15 sunscreen to apply after application of the test products. No other

I. Quantitative measurements were not determined (see Fig. 6a,b,c,d) T

products were used in the study. This design best exemplifies the way the product would be used in the marketplace. Subject selection was based on the willingness to participate in the study, with informed consent, be free of a history of cosmetic ingredient/ product sensitivity, not be pregnant or nursing, and be free of any medication that could potentially impact the study results (i.e., retinoids, anti-inflammatories, etc.). Clinical evaluations were performed at baseline, three weeks, and six weeks, including (1) high-resolution, standardized digital photography (Fuji S1, Japan) and

(2) electrical conductance measurements (Novameter Model DPM 9003, Nova Technology Corporation, Gloucester, MA, USA) for skin surface hydration; (3) blinded expert grader assessments using a 0–4 scale (0 = no change, 1 = 25% improvement, 2 = 50% improvement, 3 = 75% improvement, 4 = 100% improvement) were made via analysis of high-resolution digital photographs in a blinded scenario for skin roughness/dryness, fine lines/wrinkles, and overall global improvement in photodamaged skin. Patients were instructed to abstain from washing the facial area for a period of at least two hours prior to visits for evaluation and were acclimatized for 15 minutes in a humidity-controlled environment room prior to making Novameter moisturization measurements. Patients were also given a patient diary/questionnaire to complete designed to interpret their perceived experiences with the product and to report any skin irritations, redness, swelling, or other adverse events.

the sun-exposed periorbital/temple areas approximately 1 cm apart in randomly selected subjects from both patient subgroups (three subjects total). After formalin fixation 2-mm skin punch biopsies were paraffin embedded and cut into 5-micron sections. The paraffin sections were stained with hematoxylin-eosin (H&E) and Massons trichrome as well as immunostained with antibodies for collagen I, MMP1, IL-1b, and IL-6, and FITC conjugated antigoat antibodies used for immunofluorescence at baseline and after six weeks.

Study results

After six weeks of use of the 1.0% idebenone formulation, twice daily, a 26% reduction in skin roughness/dryness was observed, a 29% reduction in fine lines/wrinkles, a 37% increase in skin hydration, and an overall 33% improvement in global assessment of photodamaged skin was observed. For the 0.5% idebenone formulation, a 23% reduction in skin roughness/dryness was observed, a 27% reduction in fine lines/wrinkles, a 37% increase in skin hydration, and an overall 30% improvement in global assessment of photodamaged skin was observed (see Fig. 4). Before and after photography recorded a visible improvement in periorbital rhytides and pigment dyschromia from both sets of subjects receiving either the 0.5% or 1.0% formulation (see Fig. 5a,b,c,d). H&E- and Massons-stained slides demonstrated general improvement in epidermis as well as some increase in dermal collagen. Immunofluorescence staining of skin biopsies revealed decreased staining for MMP-1, IL-6 and IL-1b, and an increase in collagen

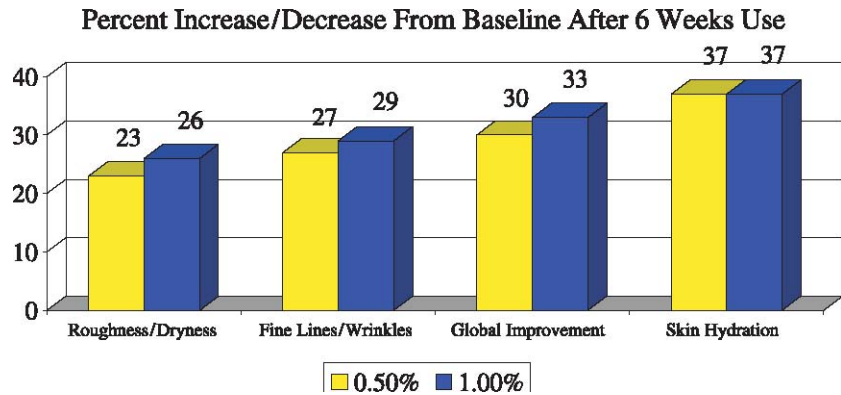


Figure 4 Data of 0.5% idebenone and 1.0% idebenone.



Figure 5 Wrinkle and pigment reduction. (a) Wrinkle reduction before and after 6-weeks' use with 1% idebenone; (b) pigment reduction before and after 6-weeks' use with 1.0% idebenone; (c) wrinkle reduction before and after 6-weeks' use with 0.5% idebenone; (d) pigment reduction before and after 6-weeks' use with 0.5% idebenone.

Discussion

included in the study, the main scope was to evaluate the

before and after effects of the products (i.e., baseline values The purpose of the testing presented was to evaluate the obtained for each individual was considered the control efficacy of two products, one containing 1% idebenone for the effects observed) and not to just specifically evaluate and the other 0.5% idebenone, pre- and post-treatment the benefits of the ingredient idebenone. Although one cannot in photodamaged skin. Although no vehicle control was definitively assign all the clinical benefits to idebenone, it

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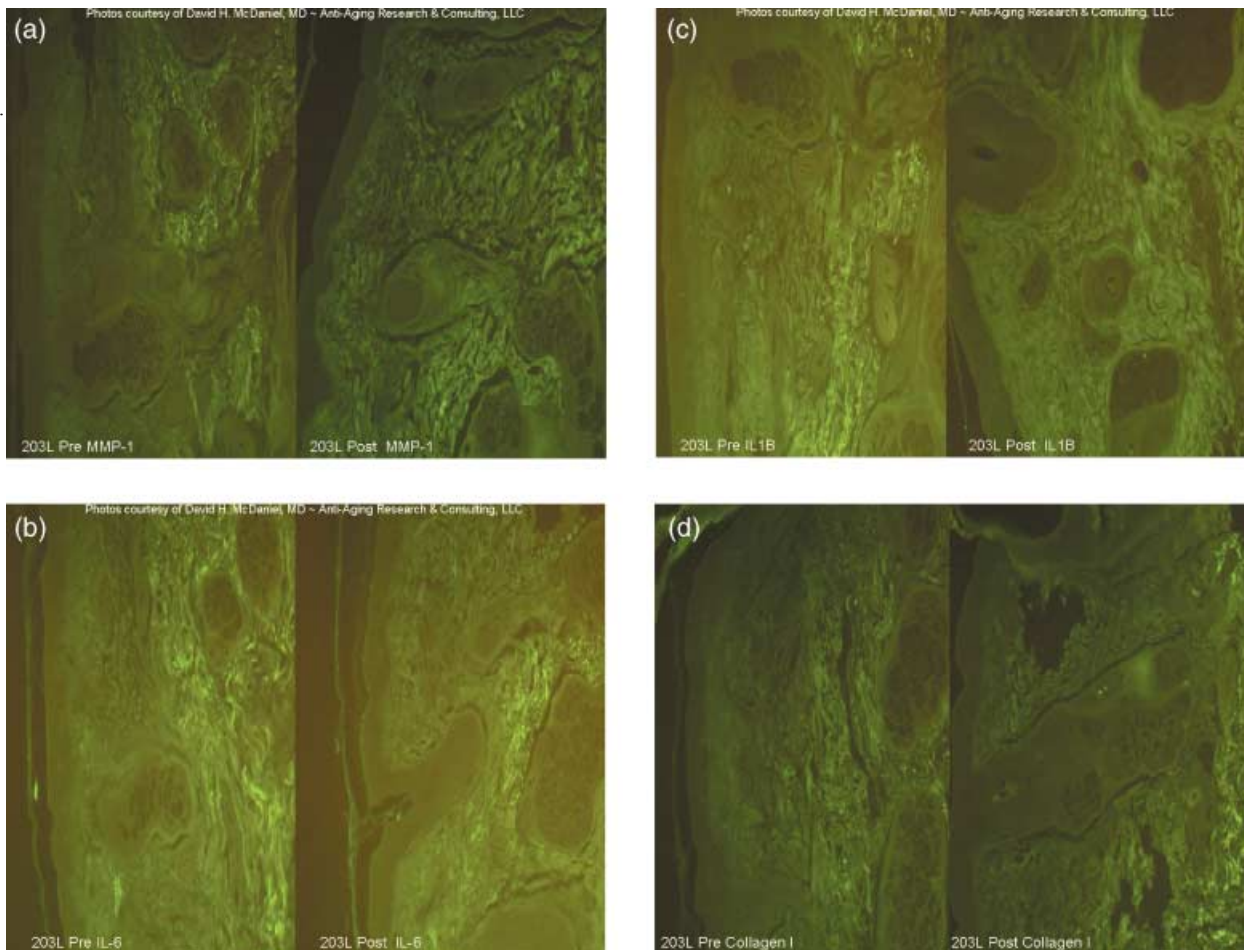


Figure 6 Immunofluorescence biopsy. (a) Before and after staining for MMP-1; (b) before and after staining for IL-6; (c) before and after staining for IL-1b; (d) before and after staining for collagen I.

was felt that the moisturization effects noted (37% for both products) appear to be associated more with the base and not with idebenone itself and that the decrease in skin roughness/dryness maybe a combination of the two. Conversely, a general moisturizer similar to what was employed in this study (i.e., free of vitamins, antioxidants, AHAs, BHAs, etc.) had not been documented to produce histological changes associated with collagen production and/or modulation of inflammatory markers. The biopsy results suggest that the mechanism of action of idebenone is to inhibit at least two free radical-mediated degradation pathways that lead to premature aging of the skin. Therefore, we believe that the results observed for the reduction in lines/wrinkles and the overall improvement in photodamaged skin are directly related to idebenone and not the base. In summary, both formulations appear to be effective in the treatment of photodamaged skin. The 1.0% idebenone formulation was about 10% more

effective overall but also delivered more expedited results. Additional clinical trials are currently being planned to go on understanding of the antioxidant benefits and mechanism of actions associated with idebenone.

Conclusion

The overall results of this clinical trial suggest that idebenone is a promising new active ingredient for topical skincare therapy of aging skin.