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# Journal of Cosmetic Dermatology

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pp 10–17



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# Idebenone: a new antioxidant – Part I. Relative assessment of oxidative stress protection capacity compared to commonly known antioxidants

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## Summary

Topical applications of skin care products containing antioxidants have become increasingly popular. Numerous studies have elucidated the biological effects of these substances. General antiaging effects, anti-inflammatory properties, photoprotective properties, and prevention of ultraviolet (UV) immunosuppression have been documented. However, a standardized method to characterize and compare the properties and oxidative stress protection capacity of antioxidants was lacking. A multistep *in vitro* process utilizing a variety of biochemical and cell biological methods combined with *in vivo* studies was designed to compare the oxidative stress protective capacity of commonly used antioxidants. Data were presented for L-ascorbic acid, dl-alpha-tocopherol, kinetin, dl-alpha lipoic acid, ubiquinone, and idebenone. Methods included using UV-induced radical trapping/scavenging capacity measured by photochemiluminescence, pro-oxidative systems (LDL-CuSO<sub>4</sub>, microsome-NADPH/ADP/Fe<sup>3+</sup>) with measurement of primary and secondary oxidation products, UVB irradiation of human keratinocytes, and *in vivo* evaluation, using the human sunburn cell (SBC) assay. Correlation and trends between *in vitro* and *in vivo* results were established, and the standardized test protocol was used to quantify oxidative stress protection capacity of antioxidants. Summarizing and totaling the data equally weighted for each oxidative stress study, the overall oxidative protection capacity scores of 95, 80, 68, 55, 52, and 41 were obtained for idebenone, dl-alpha tocopherol, kinetin, ubiquinone, L-ascorbic acid, and dl-alpha lipoic acid, respectively. The higher the score, the more effective the overall oxidative stress protection capacity of the antioxidant became. This multistep protocol may serve as a standard in investigating and comparing new putative antioxidants for topical use as well as a valuable tool to assess the anti-inflammatory properties, photoprotective properties, and prevention of UV immunosuppression of topical antioxidants.

**Keywords:** idebenone, antioxidant, aging

## Introduction

Ultraviolet radiation (UVR)-generated reactive oxygen species (ROS) and DNA photodamage play a critical role in the process of extrinsic environmentally induced aging (photoaging) and photocarcinogenesis.<sup>1</sup> In addition to the well-known long-term effects such as

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Accepted for publication January 26, 2005

immunosuppression and skin cancer, photo-oxidative damage leads to alterations of cells and structural macromolecules of the dermal connective tissue contributing significantly to photoaging with its clinical appearance of wrinkle formation, laxity, and pigment dyschromias.<sup>2</sup> The skin is constantly exposed to a pro-oxidative environment such as UVR and air pollutants. The skin is equipped with various antioxidant defense systems constituting a complex antioxidant network.<sup>1,3</sup> Whereas UVB can damage DNA, proteins, and lipids directly, UVA is believed to act largely via oxidative processes.<sup>4</sup> Increased exposure to exogenous sources and/or endogenous production of ROS can provoke an imbalance of the fragile pro-oxidant–antioxidant equilibrium, resulting in oxidative damage of lipids, proteins, and DNA.<sup>5,6</sup> For example, the superoxide ( $O_2^-$ ) radical can cause the “common deletion” mutation in mitochondrial DNA, which can be found in high numbers in photo-damaged skin.<sup>7</sup> According to the mitochondrial theory of aging, nonrepaired damage of mitochondrial DNA and unstable electron transfer cause an important loss of mitochondrial function in correlation with progression of age. Topical application of antioxidants is used to optimize the cutaneous antioxidative capacity and to limit ROS-induced skin damage. Numerous *in vitro* and *in vivo* studies have demonstrated specific antioxidative capacity as well as their photoprotective properties.<sup>6,8</sup> Antioxidants applied topically before UV-irradiation on animal and human skin diminished UVA-induced polymorphous light eruption, psoralen + ultraviolet A (PUVA)-induced erythema, and sunburn cell formation.<sup>9</sup> However, a standardized method to characterize and compare the complex properties and effects of topical antioxidants is lacking. In this study, for the first time, a variety of biochemical and cell biologic methods are combined with *in vivo* studies in a protocol to compare protective capacity of commonly used antioxidant ingredients. The *in vivo* method was included to assess real biological effects in living tissue, as human skin itself contains lipophilic antioxidants such as vitamin E (tocopherols and tocotrienols), ubiquinones (coenzyme Q), carotenoids, and lipoic acid, as well as the hydrophilic antioxidants, vitamin C (ascorbate), uric acid (urate), superoxide dismutase (SOD), and glutathione. Reduction and oxidation (redox) cellular reactions couple these antioxidants in a network together through a complex concerted action in which the antioxidants are partly recycled by one another.<sup>5</sup>

For this multistep protocol, the following compounds were tested: L-ascorbic acid (vitamin C), kinetin (a plant derivative), dl-alpha tocopherol (vitamin E), dl-alpha lipoic acid, ubiquinone (CoEnzyme Q-10), and idebenone, a lower molecular weight analog of CoEnzyme

Q-10 that showed potent radical scavenging capacity and cell protection properties in previous studies.<sup>10</sup> Five independent biochemical, cell-biologic, and *in vivo* methods were combined to determine the antioxidant capacity of the single substances under different conditions in order to demonstrate their overall performance. The studies conducted were as follows:

Study 1. Radical scavenging capacity measured by photochemiluminescence: Utilizes Photochem®, a device that offers fast and reliable chemiluminometric assessment of the general antioxidative capacity of substances in their ability to scavenge free radicals via the measurement of radicals generated (or lack thereof) through their reaction with luminol and subsequent light emission.

Study 2. Low density lipoprotein (LDL) pro-oxidative system measuring primary oxidation by-products: assessment of antioxidant ability to protect LDL stressed with copper sulphate ( $CuSO_4$ ) oxidative system. The  $CuSO_4$ -LDL system was used to evaluate the protection of lipid bulks over time measuring the primary by-products of lipid peroxidation – the highly reactive and cytotoxic lipid hydroperoxides.

Study 3. Microsome pro-oxidative system measuring secondary oxidation by-products: assessment of antioxidative ability to protect microsomal membrane stressed with NADPH/ADP/ $Fe^{3+}$ -oxidative system measuring secondary oxidative by-products (malondialdehyde – MDA equivalents) utilizing the thiobarbituric acid-reactive substances (TBARS) method.<sup>11</sup> Antioxidants protecting bulky lipids, such as LDL, are not necessarily good protectors of cell membranes as a result of their hydrophilic/lipophilic bilayer composition. Therefore, the pro-oxidative NADPH/ADP/ $Fe^{3+}$ -microsome system was used as an *in vitro* model system more closely resembling natural cellular biological systems. Oxidation of cell membranes leads to serious consequences in altering cell membrane fluidity and cell function.

Study 4. UVB irradiation of keratinocytes measuring DNA damage: assessment of DNA damage in cell culture experiments under pro-oxidative conditions (UVB irradiation of human keratinocytes) by measuring the positive cells for antithymine dimer antibodies. This experiment is thought to reflect a direct correlation to the *in vivo*-occurring DNA cross linking damage following UVB exposure and the protection of such nuclear damage by antioxidants.

Study 5. UVB irradiation of human skin measuring damage by formation of sunburn cells (SBC): Exposure to UVR can cause damage of epidermal cells, resulting in the formation of sunburn cells.<sup>12</sup> Because sunburn cells can be enumerated, their formation provides a relatively sensitive and quantitative measure of the extent of UVR damage to the epidermis.

## Methods, materials, and results

### Study 1. Radical scavenging capacity measured by photochemiluminescence

The individual antioxidant capacity of the putative antioxidant substances was estimated by the Photochem® system (Analytik Jena AG, Jena, Germany, and Analytik Jena USA, Inc., TX). The system combines the generation of radicals through photochemical excitation with highly sensitive luminometric detection via the radical reaction with luminol to produce measurable light emission. Samples are diluted with premade buffers (standardized kits) and applied to the device. The relative antioxidative capacity is determined by comparison to a standardized blank (without antioxidants) and a standard provided with the kit. The ACL-kit (integral antioxidative capacity of lipid-soluble substances) and the ACW-kit (integral antioxidative capacity of water-soluble substances) were used. Antioxidant concentrations effective to eliminate radical formulation were established for each antioxidant as indicated in Table 1.

#### Results

Idebenone, dl-alpha-tocopherol, and L-ascorbic acid all demonstrated effective neutralization of radicals as measured by photochemiluminescence at a relatively low concentration of 10 nmol/L. Ubiquinone was somewhat less effective, requiring 100 nmol/L for effective concentration, kinetin required 1,000 nmol/L for effective concentration, and alpha lipoic acid was ineffective in this system (see Table 1).

### Study 2. LDL pro-oxidative system measuring primary oxidation by-products

#### Isolation of LDL

Low density lipoproteins ( $d = 1.019\text{--}1.063$  kg/L) were isolated in clean Beckman one-way Quick-Seal© Tubes (Beckman Coulter Inc., Palo Alto, CA) by ultracentrifugation from pooled plasma of healthy donors using an established protocol. After isolation, LDLs were extensively dialyzed against a degassed and nitrogen-saturated tris-hydrochloric (HCl) buffer (5 mmol/L, pH 7.4) containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA). Before oxidation by  $\text{CuSO}_4$ , EDTA was removed from LDL by dialysis against a tris-HCl buffer (5 mmol/L, pH 7.4) without added EDTA.

trifugation from pooled plasma of healthy donors using an established protocol. After isolation, LDLs were extensively dialyzed against a degassed and nitrogen-saturated tris-hydrochloric (HCl) buffer (5 mmol/L, pH 7.4) containing 1 mmol/L ethylenediaminetetraacetic acid (EDTA). Before oxidation by  $\text{CuSO}_4$ , EDTA was removed from LDL by dialysis against a tris-HCl buffer (5 mmol/L, pH 7.4) without added EDTA.

#### Incubation LDL with the pro-oxidant system Ham's F-10/ $\text{CuSO}_4$

The LDL oxidation was achieved by incubating (37 °C, 95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ) 1 g of LDL protein/L with and without the putative antioxidant substances (at equivalent 100  $\mu\text{mol}$  concentrations) in 2 mL of serum-free Ham's F-10 medium (BioSource International, Camarillo, CA) in the presence of 20  $\mu\text{mol/L}$   $\text{CuSO}_4$  for the times indicated in the figure legends.

#### Measurement of lipid hydroperoxides

Lipid hydroperoxides were determined with the Cayman Lipid Peroxidation (LPO) Assay Kit (Cayman Chemical, Ann Arbor, MI) which measures the hydroperoxides directly utilizing the redox reactions with ferrous ions to produce ferric ions which can be detected using thiocyanate ion as the chromogen. The antioxidative effect of the substances is shown as percentage compared to the Blank (incubation without addition of antioxidants).

#### Results

Kinetin and idebenone demonstrated a consistent protection against lipid peroxidation over 24 h. Other substances like ubiquinone, lipoic acid, and ascorbic acid showed only a comparatively short-lasting protective efficiency (see Fig. 1).

### Study 3. Microsome pro-oxidative system measuring secondary oxidation by-products

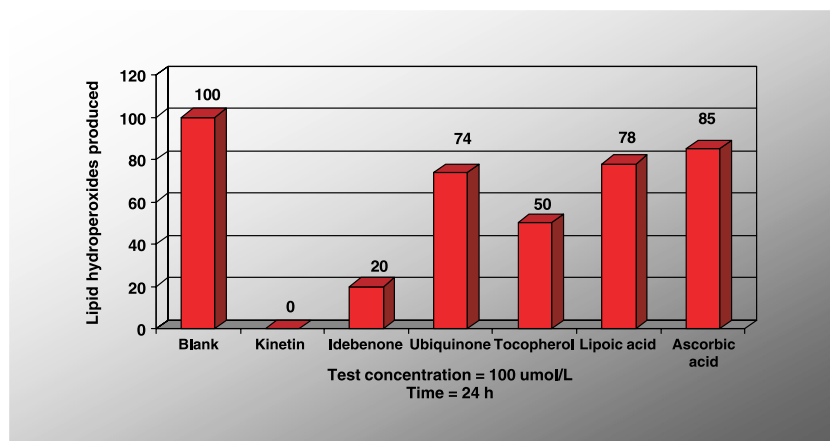
#### Preparation of microsomes

Livers were obtained from male Wistar rats weighing between 250 and 400 g. Tissue was homogenized in 50 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid (HEPES), 250 mmol/L sucrose buffer, pH 7.4, containing 150 mmol/L potassium chloride (KCl) and 500  $\mu\text{mol/L}$  EDTA using a Kinematica Polytron PT3000 (Brinkmann Instruments, Westbury, NY) homogenizer. Microsomal vesicles were isolated by removal of the nuclear fraction at 8,000  $g$  for 10 min at 4 °C and removal of the mitochondrial fraction at 18,000  $g$  for 10 min at 4 °C using a Beckman L8-55 ultracentrifuge and a 50Ti-13 rotor. The microsomal fraction was sedimented at 105,000  $g$  for 60 min at 4 °C. The pellet was

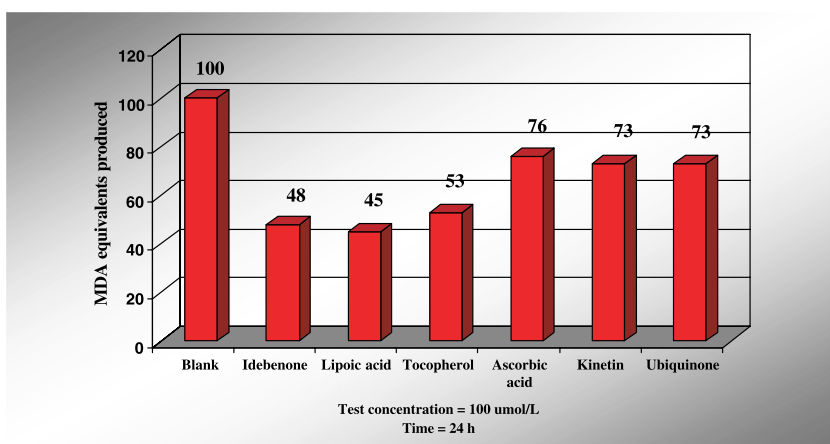
**Table 1** Radical scavenging capacity measured by photochemiluminescence.

Antioxidant substance	Effective concentration (nmol/L)
Idebenone	10
Ascorbic acid	10
Tocopherol	10
Ubiquinone	100
Kinetin	1000
Lipoic acid	> 1000 (not detectable)

**Figure 1** Low density lipoprotein (LDL) pro-oxidative system measuring primary oxidation by-products (lipid hydroperoxides).



**Figure 2** Microsome pro-oxidation system measurement of secondary oxidative by-products (MDA equivalents).



washed once in 50 mmol/L HEPES and 150 mmol/L KCl, pH 7.4, and collected again at 105 000 *g* for 30 min. The resulting microsomal pellet was resuspended in HEPES/KCl, pH 7.4, by careful sonication in ice and stored in portions (10 mg protein/mL) at  $-80^{\circ}\text{C}$  until use.

#### *Incubation of microsomes with the pro-oxidant system NADPH/ADP/Fe<sup>3+</sup>*

The microsomal preparations were incubated in the presence of the pro-oxidant system NADPH/ADP/Fe<sup>3+</sup>, consisting of 0.20 mmol/L NADPH, 50 mmol/L ADP, and 0.25 mmol/L FeCl<sub>3</sub> in HEPES/KCl buffer (150 mmol/L KCl, 50 mmol/L HEPES) with and without the putative antioxidant substances. Oxidation of 1-mL aliquots containing 1 mg of protein was started at 37 °C by the addition of NADPH and was stopped with EDTA (10 mmol/L) after the times indicated in the figure legends. Control incubations without the pro-oxidant system were performed at 37 °C in the presence of EDTA. All antioxidants were dissolved in water or ethanol and added to the incubations at equivalent 100 μmol concentrations.

#### *Measurement of secondary oxidation products, MDA equivalents or TBARS method*

Microsomal preparations (500 μL) were mixed with 1 mL of thiobarbituric acid (0.67 g/100 mL, 0.05 mol/L sodium hydroxide – NaOH). After the addition of trichloroacetic acid (50% w/v), the samples were heated to 90 °C for 30 min. After cooling and extraction of the samples with 1 mL of butanol, the absorbance of the butanol phase was determined spectrophotometrically at 532 nm. For quantification, an external standard curve was prepared using 1,1,3,3-tetraethoxypropane, which yields MDA. The antioxidative effect of the substances is shown as percentage compared to the control (incubation without addition of antioxidants).

#### *Results*

Lipoic acid and idebenone showed the most effective protection against oxidation of the cell membrane lipids. Kinetin, which showed favorable results in protecting bulky lipids (LDL), showed only a weak protective effect in the ability to protect microsomal membranes (see Fig. 2).

#### Study 4. UVB irradiation of keratinocytes measuring DNA damage

##### *Keratinocyte collection*

Human primary foreskin keratinocytes (second passage) were grown in 6-well plates containing cover slips to 60% confluence in serum free medium (KGM, Clonetics; Cambrex Cooperation, E. Rutherford, NJ) containing 0.07 mM calcium chloride (CaCl<sub>2</sub>). Six hours before UVB-radiation, the medium was removed and replaced by fresh growth medium with or without the antioxidative substances. Each antioxidant concentration was 10 μmol per well.

##### *Ultraviolet light (UVB) irradiation of keratinocytes*

Keratinocyte cultures were irradiated with a single dose of 200 mJ/cm<sup>2</sup> UVB, using FS-20/T-12 bulbs (emission range: 280–340 nm; 305 nm max.). Immediately prior to irradiation, the medium was replaced with 1 mL sterile phosphate buffered saline (PBS) (pH 7.4, 37 °C), and after irradiation, PBS was replaced with fresh growth medium without antioxidants. The UVB exposure was quantified using a Goldilux™ Ultraviolet Radiometer (Oriental Instruments, Stratford, CT). Cells were maintained at 37 °C (5% CO<sub>2</sub>) for 1 h until fixation with paraformaldehyde (PFA).

##### *Fixation and nuclear thymine–dimer staining of keratinocytes*

Cells were fixed with 4% PFA in PBS for 30 min at room temperature (RT), washed with PBS and permeabilized by incubation with EtOH/PBS (90/10; v%/v%) for 30 min at ~10 °C. After fixation and permeabilization, cells were washed twice with PBS containing 1% of bovine serum albumin (BSA). They were then incubated for 30 min with 10 μg/mL antithymine dimer Ab (clone KTM53; Kamiya Biomedical Company, Seattle, WA) at RT. After the incubation period, the cells were washed twice with PBS-BSA and incubated for 30 min with 20 μg/mL secondary fluorescein-isothiocyanate (FITC)-conjugated antimouse immunoglobulin G (IgG) at RT. After the incubation with the secondary antibody, cells were washed twice with PBS-BSA and fixed again with 4% PFA for 15 min at RT. Slides were analyzed by confocal microscopy.

##### *Results*

This experiment is thought to reflect the *in vivo* occurring DNA damage following UVB exposure and the protection of such nuclear damage by antioxidants. The results (see Table 2) have to be seen as approximate estimations of the occurrence of nuclear thymine dimer photo products. Idebenone provided the highest level of inhibition.

**Table 2** UVB irradiation of keratinocytes measuring DNA damage.

Antioxidant substance	Positive cells after UVB radiation*	Inhibition of photoproduct generation (protective effect)
No radiation control	0%	100%
Idebenone	29%	45%
Ascorbic acid	34%	36%
Kinetin	34%	36%
Tocopherol	35%	34%
Ubiquinone	51%	4%
Lipoic acid	53%	0%
200 mJ/cm <sup>2</sup> UVB radiation	53%	0%

\*Percentage of positive cells (above threshold) in three fields (counted cell number ~120–150).

#### Study 5. UVB irradiation of human skin measuring damage by formation of sunburn cells (SBC)

##### *Treatment*

All applications were made to a 5 × 10 cm area site over the mid-back region once a day for 2 weeks. Each putative antioxidant was applied to five (*n* = 5) healthy adult volunteers between the ages of 18 and 60. All antioxidants were dissolved in ethanol/water at 0.5% w/w concentrations. Additionally, one test site was left untreated and served as a control. Approximately 10 min after the last application, test sites were irradiated to 1.5 minimal erythema dose (MED) of UVB light, a shave biopsy taken and prepared histologically, and the number of sun burn cells (SBC) evaluated microscopically per high power field.

##### *Light source*

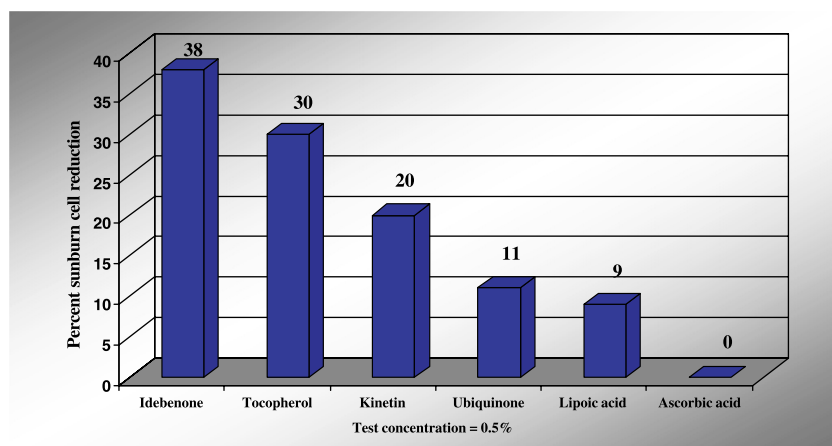
The light source used was a 150-W xenon arc solar simulator equipped with a UV reflecting dichromic mirror and a 1-mm thick Schott WG-320 (BES Optics Inc., W. Warwick, RI) filter to produce simulation of the solar spectrum. A 1-mm thick UG5 filter was added to remove reflected heat and remaining visible radiation.

##### *Minimal Erythema Dose (MED) determination*

The MED for each subject was determined by exposing a circle 1 cm in diameter to untreated areas to a series of exposures in 25% dose increments from the solar simulator. The MED was defined as the time of exposure required to produce a minimally perceptible erythema 20 ± 4 h after exposure.

##### *Biopsies*

Approximately 10 min after the last topical application of the putative antioxidant, a circular area measuring 1 cm



**Figure 3** UVB irradiation of human skin measuring DNA damage by formation of SBC.

in diameter was exposed to a single dose of 1.5 MED using the solar simulator. Approximately 20 h later, a shave biopsy (~4 × 4 mm) was obtained from each irradiated and untreated control site following injections of a local anesthetic (lidocaine). The skin specimens were immediately fixed in 10% buffered formalin.

#### Histology

The fixed specimens were processed routinely, embedded in paraffin, and then sectioned and stained with hematoxylin-eosin. The numbers of SBC were determined in at least 12 sections at 50- $\mu$  intervals. A minimum of 70 high power fields (HPF) was counted from each biopsy, and the average number of SBCs per HPF determined. All specimens were counted in a blinded manner.

#### Results

Figure 3 expresses the photoprotective benefits of the antioxidants tested based on the percent change over baseline (delta percent) for the number of SBC per high power field. Idebenone was the most effective antioxidant in the study in its ability to protect human skin from sunburn cell formation post-UVR exposure.

#### Discussion

Previous studies comparing different antioxidants for use in topical applications primarily focused on certain biochemical or cell-biologic aspects of those substances. Because the currently popular topical antioxidants are of very heterogeneous structure and origin (vitamins, flavonoids, coenzymes, etc.), a protocol to compare their properties should consist of a variety of methods aiming to elucidate the overall picture regarding their specific

antioxidative capacities. In this study, a multistep protocol is presented to allow the comparison of different antioxidants regarding their usefulness in topical applications. This combination of biochemical, cell-biologic, and *in vivo* methods allows the determination of various independent aspects of antioxidant substances, such as anti-inflammatory properties, photoprotective properties, or protection of cell membranes. The results demonstrate the diversity of actions and the value of utilizing a combination of entirely different methods when comparing the relative efficacy of antioxidant activity. Kinetin for example showed a very weak antioxidant effect when evaluated by chemiluminometric detection of antioxidative capacity yet showed the strongest effect in the LDL/CuSO<sub>4</sub>-oxidation system. Another example is lipoic acid which showed a strong effect in the microsome-NADPH/ADP/Fe<sup>3+</sup>-system while showing minimal response in every other method employed. Even tocopherol, which showed good results in most experiments, revealed a weakness regarding lipid protection over time (LDL/CuSO<sub>4</sub>-oxidation system). This is most likely because its pro-oxidative metabolites appear through reduction of radicals by hydrogen donation. The implementation of an *in vivo* approach employing the human SBC assay gave crucial additional clinical information, as the capacity of each compound to penetrate the upper skin layers may vary. These results confirm that favorable *in vitro* results do not necessarily reflect the *in vivo* situation. An example thereof is ascorbic acid which surprisingly had no protective effect (at the concentration tested) on *in vivo* SBC formation. Idebenone, while to date had demonstrated only the ability to protect against ROS-mediated damage in organ preservation solutions and to treat Alzheimer's disease,<sup>13</sup> showed a strong overall performance throughout all experiments conducted.

**Table 3** Global relative antioxidant activity: total oxidative stress protection capacity scores (environmental protection factor; EPF of common antioxidants).

Test	Idebenone	Tocopherol	Kinetin	Ubiquinone	Ascorbic acid	Lipoic acid
Sun burn cell assay	20	16	11	6	0	5
Photochemiluminescence	20	20	10	15	20	5
Primary oxidative products	16	10	20	5	3	4
Secondary oxidative products	19	17	10	12	12	20
UVB irradiated keratinocytes	20	17	17	17	17	7
Total points (EPF score)	95	80	68	55	52	41

### Establishing a standardized way to summarize results

The scoring system introduced in this study is designed to provide, for the first time, a standardized comparison of the protective capacity of different antioxidant substances used in topical applications against oxidative stress. Five tests, which were equally weighted at 20 points each, allowed for the maximum highest possible score of 100 points. Using this scale, the higher score indicates higher oxidative stress protection capacity of the antioxidant. Equal weight was given to the antioxidant's performance in each study because each study tested the antioxidant's ability to protect against a unique different set of oxidative stress parameters. Without knowing the direct correlation of each study to actual living biological systems (with one exception, the SBC study was conducted *in vivo*), the equal weight approach was selected for the initial introduction of this concept. Future experiments and clinical correlation should allow refinement of this concept and the weighting may be adjusted, if data warrant, for optimal clinical correlation. For example, it is known that the superoxide radical, a natural by-product of metabolic energy production, causes serious deleterious effects to living cells if not quenched, neutralized, or reduced almost immediately after production. In study 1, we tested the ability of the antioxidant to suppress superoxide radical formation. It is also known that lipid peroxidation is a major problem in biological systems. In study 2 we assessed the antioxidants' ability to suppress lipid oxidation. Protecting cell membrane oxidation is of paramount importance to living biological systems because the cell membrane is the cell's first line of defense against oxidation. In study 3, we assessed this protective parameter. Because UV light is known to be the predominant cause of premature aging of the skin, the antioxidants' ability to protect against UV-induced oxidative stress was tested under both *in vitro* (study 4; UVB irradiation of keratinocytes measuring DNA

damage) and *in vivo* (study 5; UVB irradiation of human skin measuring damage by formation of SBC) test methods. Therefore, overall, equal weighting of study results seems to be most appropriate at this time. To assign values, the active antioxidant that demonstrates the greatest benefit for the test conducted became the standard for the study, and received 20 points. The remaining antioxidants were assigned a percentage of the 20 points based on their efficacy relationship to the highest scoring antioxidant in each independent study.

Example: idebenone produced the greatest benefit in the SBC assay, a 38% reduction in SBCs. Therefore, it became the standard and received 20 points. Tocopherol was second, producing a 31% reduction. To determine the relative activity (efficacy) of tocopherol to the standard, idebenone, one calculates  $31/38 \times 100 = 82\%$  relative activity. Therefore, tocopherol would receive 82% of the 20 points or 16 points (rounded to the nearest whole number).

Because the photochemiluminescence assay results are expressed as the lowest effective concentration, and a base 10 serial dilution was used, the antioxidant scores were assigned as follows: 10 nmol/L = 20; 100 nmol/L = 15; 1000 nmol/L = 10; > 1000 nmol/L = 5.

The "relative value" of each of the five tests was arbitrarily assigned equal weight. That is, each test contributed equally to the antioxidant scale. Further clinical testing in the future may allow additional refinement of this scoring system and weight. The overall scores, and thus relative oxidative stress protection capacity of the respective antioxidants tested, are summarized in Table 3.

### Conclusion

These studies compared the protective capacity of five commonly used antioxidant ingredients and one novel new antioxidant for skin care, idebenone, in both *in vitro* and *in vivo* methods. A standardized testing protocol that quantifies the oxidative stress protection capacity of the

substances studied was developed, and a scoring system to compare relative activity was presented.

This quantitative scoring system to assess the oxidative stress protection capacity of an antioxidant could be the basis for an antioxidant ingredient performance rating system that consumers could easily understand. Similar in analogy to sun protection factor (SPF) or immune protection factor (IPF),<sup>14</sup> the antioxidant protection factor or environmental protection factor (EPF) could be used to assess the relative strength of antioxidants in their ability to protect against oxidative stress. Reviewing the summation of all study results presented (Table 3), one compound, idebenone, appears as a powerful antioxidant most consistently throughout all experiments. Although this potent antioxidant is relatively unknown to dermatology today, idebenone may represent a promising new agent for topical skin care protection. Further research is currently being conducted to establish its *in vivo* ability to protect human skin from oxidative stress and assess its efficacy in the treatment of photodamaged skin.

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