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In vivo photoprotective effect of β-bis(carboxyethyl)germanium sesquioxide

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Synopsis

In this paper *in vivo* radical scavenger activity of β-bis(carboxyethyl)germanium sesquioxide (CEG) was investigated. CEG aqueous solutions were topically applied to healthy human volunteers before and after skin exposure to UVB radiation. Skin erythema was monitored by calculating erythema index values from the skin spectral data obtained using a reflectance spectrophotometer. The results of these experiments indicated that CEG was not able to inhibit UVB-induced skin erythema both in the pretreatment and in the posttreatment protocol. In order to assess if the lack of CEG photoprotective activity was due to poor CEG *in vivo* skin permeation, we tested CEG aqueous formulations containing a skin penetration enhancer such as dimethylisosorbide (DMI). CEG aqueous solutions containing DMI were able to reduce UVB-induced skin erythema when applied before skin exposure to UV radiation, but they were ineffective when topically applied after UVB irradiation.

INTRODUCTION

The use of radical scavengers to prevent UV-induced skin damage has recently been met with considerable interest. Bissett *et al.* (1) and Trevithick (2) reported that superoxide-scavenging antioxidants, such as tocopherol and tocopheryl acetate, decreased UVB skin damage after topical application in mice. Other authors (3) suggested that topical free radical scavengers, like SOD, could exert photoprotective effects against ultraviolet radiation-induced cutaneous damage.

β-Bis(carboxyethyl)germanium sesquioxide (Arlamol GEO®, CEG) (Figure 1), a compound of very low toxicity, has been shown to have antioxidant/radical scavenger properties in *in vitro* studies (4,5). Presently CEG is used in anti-aging and skin-protective products in Europe and Asia and as a dietary supplement in several Asian and European countries. However, to date in the literature no data have been reported on a CEG protective effect against UV radiation.

Therefore, in this study the *in vivo* radical scavenger activity was assessed by determining the ability of CEG to reduce UVB-induced skin erythema on healthy human volunteers. CEG aqueous solutions were topically applied before and after skin exposure to UVB radiation, and the subsequent skin erythema was monitored by calculating ery-

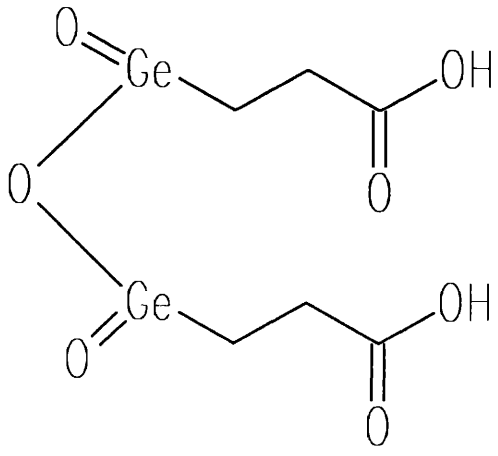

Figure 1. β-Bis(carboxyethyl)germanium sesquioxide (CEG) chemical structure.

thema index values from the skin spectral data obtained using a reflectance spectrophotometer.

MATERIALS AND METHODS

MATERIALS

In this study the following compounds were used (trade names and manufacturers in parentheses): β-bis(carboxyethyl)germanium sesquioxide (Arlamol GEO®, CEG) and dimethylisosorbide (Arlasolve DMI®, DMI) (ICI Surfactants, Belgium) and [^{3}H]water (specific activity 5 mCi/ml) (Amersham, U.K.). All other substances were of analytical grade.

IN VIVO PHOTOPROTECTIVE EFFECT EVALUATION

UVB-induced skin erythema was monitored by means of a reflectance visible spectrophotometer, X-Rite model 968, having 0° illumination and a 45° viewing angle, as previously reported (6). The instrument was calibrated with a supplied white standard traceable to the National Bureau of Standards' perfect white diffuser. The spectrophotometer was connected to an IBM PS2 50 computer, which performed all color calculations from the spectral data by means of the Spectrostart program supplied with the instrument. Reflectance spectra were obtained over the wavelength range of 400–700 nm using illuminant C and 2° standard observer.

In vivo experiments were performed on six healthy volunteers (both sexes) of skin types II and III, with an average age of 31 ± 9 years. All the volunteers were fully informed of the nature of the study and the procedures involved, and they gave their written consent. The subjects did not suffer from any ailments and were not on medication at the time of the study. They rested for 15 min. prior to the experiments, and room conditions were set at 22 ± 2°C and 40–50% relative humidity.

Skin erythema was induced by UVB irradiation using an ultraviolet lamp, model UVM-57 (UVP, San Gabriel, CA), that emitted in the range of 290–320 nm, with an

output peak at 302 nm. The flux rate measured at the skin surface was 0.80 mW/cm^2. For each subject, the minimal erythema dose (MED) was determined preliminarily, and an irradiation dose corresponding to the double of the MED was used throughout the study.

For each subject, eight sites on the ventral surface of one forearm and six sites on the other were defined using a circular template (1 cm^2) and demarcated with permanent ink. Two different formulations of CEG were used: a CEG-saturated solution in distilled water and a CEG-saturated solution in water:Arlasolve DMI 50:50. A solution consisting of water:Arlasolve DMI 50:50 (without CEG) was also used to assess the DMI effect on UVB-induced skin erythema. For each subject, two skin sites were left untreated but exposed to UVB radiation (control).

The protocol consisted of two series of experiments (6). In the first series (pretreatment protocol), the formulations tested were applied randomly for 3 h on the skin sites of one forearm using a Hill Top chamber (Hill Top Research Inc., Cincinnati, OH) whose cotton pad was saturated with 150 μl of the formulation being tested. After 3 h, the chambers were removed, the skin surfaces were gently washed with water to remove the formulation, and each pretreated site was exposed to UVB irradiation.

The second series of experiments was simultaneously performed on the other forearm of the same subjects. Skin sites were exposed to UVB irradiation, and then the formulations tested (150 μl) were immediately applied to the irradiated sites (using the same Hill Top chamber described above) for 6 h. After this period, the chambers and the formulations were removed. For both experimental protocols, UVB-induced erythema was monitored for 58 h using the reflectance spectrophotometer described above.

From the skin spectral data obtained, the erythema index (E.I.) was calculated using equation 1 reported by Dawson *et al.* (7):

$$\text{E.I.} = 100\left[\text{Log}\frac{1}{R_{560}} + 1.5\left(\text{Log}\frac{1}{R_{540}} + \text{Log}\frac{1}{R_{580}}\right) - 2\left(\text{Log}\frac{1}{R_{510}} + \text{Log}\frac{1}{R_{610}}\right)\right] \quad (1)$$

where 1/R is the inverse reflectance at a specific wavelength (560, 540, 580, 510, and 610 nm). E.I. baseline values were taken at each designated site before application of the formulations tested (pretreatment protocol) or before UVB irradiation (posttreatment protocol), and they were subtracted from the E.I. values obtained after formulation application at each time point, to determine Δ E.I. values. For each site, the area under the response (ΔE.I.)-time curve (AUC) was computed using the trapezoidal rule.

AUC values were inversely related to the ability of the formulations tested to inhibit UVB skin erythema. To better compare the efficacy of the different formulations tested, the percentage inhibition of UVB skin erythema (PIE) was calculated from AUC values using the following equation:

$$\text{Inhibition \% (PIE)} = \frac{AUC_{(C)} - AUC_{(T)}}{AUC_{(C)}} \times 100 \quad (2)$$

where $AUC_{(C)}$ is the area under the response-time curve of sites that received no treatment (control), $AUC_{(T)}$ is the area under the response-time curve of the sites treated with the formulations being tested. Statistical analysis of the results were performed using Student's t-test.

RESULTS AND DISCUSSION

UVB erythema is regarded as one of the most suitable models for studying *in vivo* skin damage after acute UV exposure (1). *In vitro* and *in vivo* studies have shown that activated oxygen species and oxygen radicals are involved both in the inflammatory response elicited by acute UV skin exposure (skin erythema) (8) and in the photoaging and carcinogenesis processes induced by chronic UV skin irradiation (9). It has therefore been suggested (1,10,11) that the evaluation of the photoprotective effect against ultraviolet light-mediated cutaneous damage can provide a useful tool to assess radical scavenger activity of topical applied compounds.

In this study, we used two different protocols for evaluating CEG ability in inhibiting UVB skin erythema: (a) skin sites were pretreated with formulations containing CEG, and then, after removal of the formulation, they were exposed to UVB radiation, and (b) skin sites were irradiated with UVB, and then the same formulations used in the pretreatment protocol were applied. The time course of erythema for skin sites treated with CEG formulations before and after UVB irradiation is reported in Figures 2 and 3, respectively. From Δ E.I. vs time plots, the area under the response (Δ E.I.)-time curve (AUC) was computed using the trapezoidal rule, and AUC values are reported in Table I. As may be noted, CEG was not able to inhibit UVB-induced skin erythema using both the pre- and posttreatment protocols since AUC values for skin sites treated with CEG aqueous solutions were not significantly different ($p > 0.05$) from those of the control (untreated sites). The lack of activity of carboxyethylgermanium from water formulations, both in the pretreatment and posttreatment protocol, could be due to: (a)

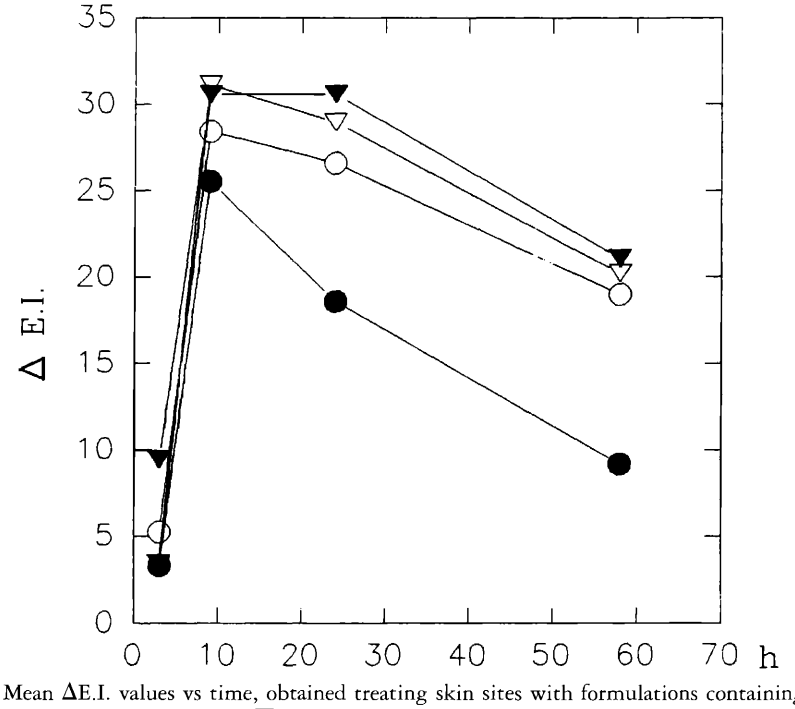

Figure 2. Mean ΔE.I. values vs time, obtained treating skin sites with formulations containing CEG before skin exposure to UVB radiation: (▼) control; (○) CEG aqueous solution; (●) CEG solution containing Arlasolve DMI; (▽) water:DMI 50:50.

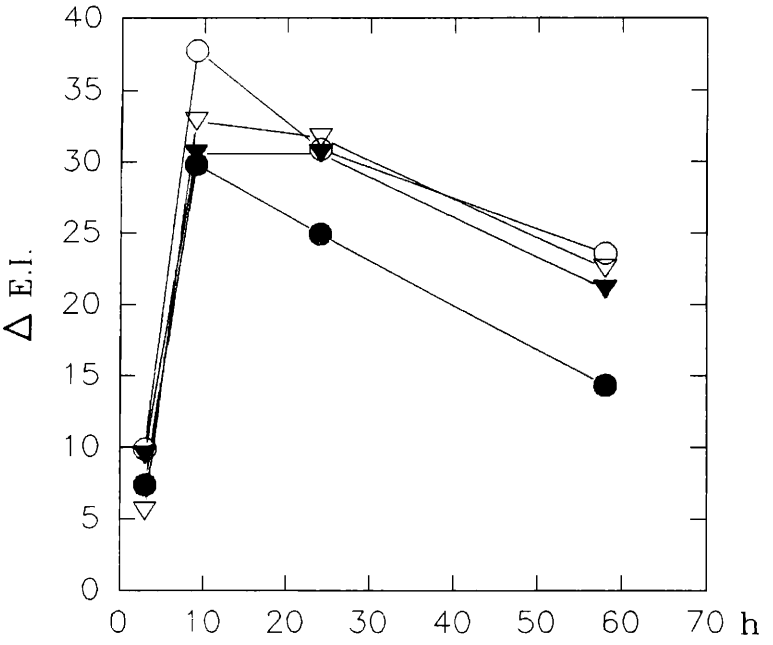

Figure 3. Mean ΔE.I. values vs time, obtained treating skin sites with formulations containing CEG after skin exposure to UVB radiation: (▼) control; (○) CEG aqueous solution; (●) CEG solution containing Arlasolve DMI; (∇) water:DMI 50:50.

rapid depletion of the active compound within the skin, and (b) poor *in vivo* percutaneous absorption of CEG, which could lead to an amount of active compound within the skin too small to exert any activity in the following inflammation process. In order to assess if the lack of CEG photoprotective activity was due to poor CEG *in vivo* skin permeation we tested CEG aqueous formulations containing DMI, a compound used by

Table I
AUC Values Obtained Treating the Skin With Formulations Containing CEG Before or After UVB Irradiation

		Posttreatment			Pretreatment		
Subject	Control	CEG[a]	CEGDMI[b]	DMI[c]	CEG[a]	CEGDMI[b]	DMI[c]
A	1482.17	1789.42	1665.15	1765.42	1263.77	1000.93	1402.37
B	1539.05	1526.39	960.96	1530.22	1004.39	854.71	1605.41
C	1378.03	1502.89	905.47	1327.51	1223.43	905.27	1133.01
D	1299.01	1368.66	1250.23	1224.36	1338.51	726.18	1208.46
E	1584.78	1702.00	1409.71	1671.63	1500.93	944.33	1678.57
F	1451.36	1599.41	939.56	1594.31	1374.68	899.83	1303.61
Mean	1455.73	1581.46	1188.51[d]	1518.91[d]	1284.28	888.54[e]	1388.57[e]
± S.D.	104.80	150.06	307.83	206.54	167.64	93.44	217.38

[a]Skin sites were treated with CEG aqueous solution.
[b]Skin sites were treated with CEG solutions consisting of water:Arlasolve DMI 50:50.
[c]Skin sites were treated with water:Arlasolve DMI 50:50 without CEG.
[d]$p < 0.03$.
[e]$p < 0.002$.

others (12,13) as a skin penetration enhancer. The formulation containing CEG and DMI was effective in reducing UVB-induced skin erythema (PIE value 38.96%) using the pretreatment protocol, while it was ineffective when applied after UVB skin exposure. To evaluate a potential DMI photoprotective effect we tested aqueous solution containing DMI alone. As shown in Table I, DMI was not able to inhibit UVB-induced skin erythema since AUC values, obtained applying DMI:water solution (50:50) before or after UVB irradiation, were not significantly different from those of the control ($p > 0.05$). This finding suggests that DMI could enhance the amount of active compound penetrated through the skin. Since DMI was only effective using the pretreatment protocol, we surmise that this enhancer needs a certain period of time to exert its enhancement effect.

To exclude, in the pretreatment experiments, a potential "sunscreen effect" of CEG in DMI:water (50:50) solution we report (Figure 4) the UV spectrum (200–400 nm) of this compound: as shown in this spectrum, there is no absorption of CEG in the 290–400-nm range. Therefore, on the basis of both lack of absorption and CEG biological activity reported in the literature (4,5), we can conclude that the CEG's radical scavenging may probably be the mechanism of the *in vitro* photoprotection activity of this compound.

In conclusion, CEG water formulations using both the pretreatment and posttreatment protocols did not inhibit UVB-induced skin erythema, while CEG formulations containing DMI were effective in inhibiting UVB-induced skin erythema only when ap-

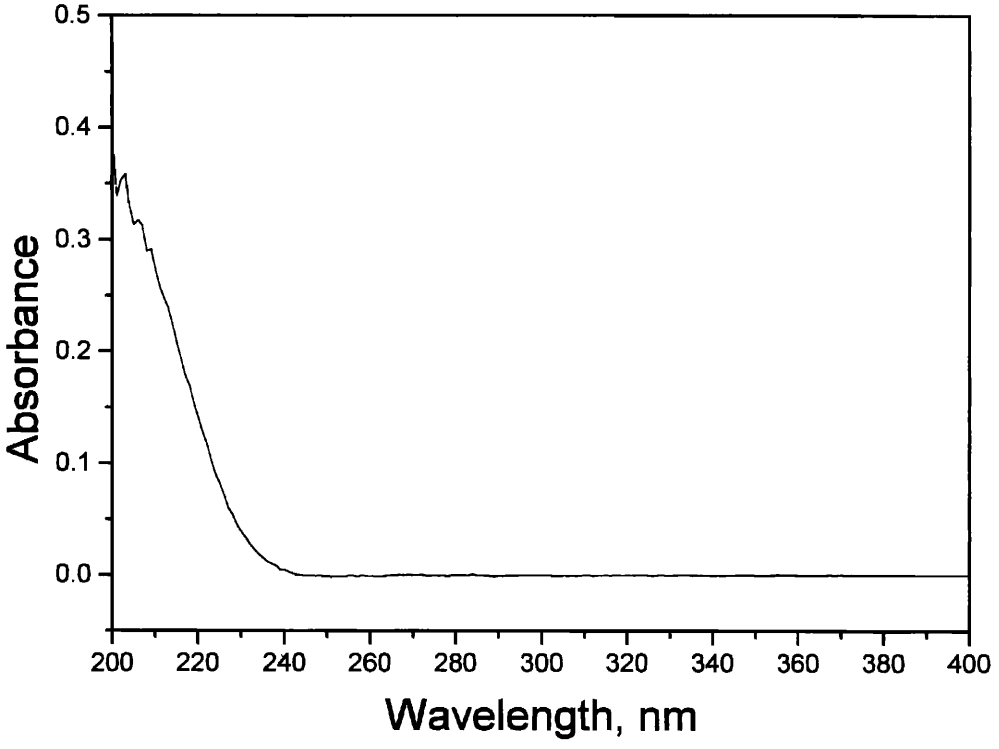

Figure 4. UV spectrum (200–400 nm) of CEG ($3.01 \cdot 10^{-3}$ M) in DMI:water (50:50).

plied before skin exposure to radiation. We conclude that topical application of CEG formulations containing DMI could be used for skin care products containing radical scavengers.

Further *in vivo* studies are planned in order to compare radical scavenger activity of CEG with that of others generally used in cosmetics and to assess the effect of different skin penetration enhancers on CEG photoprotection.

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