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INVESTIGATIVE REPORT

UVB Photoprotection with Antioxidants: Effects of Oral Therapy with d-α-Tocopherol and Ascorbic Acid on the Minimal Erythema Dose

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Ultraviolet radiation absorption is responsible for the production of free radicals in damaged cells. This side effect may be neutralized using antioxidant substances. It has been reported that ascorbic acid and d-α-tocopherol scavenge reactive oxygen species. In a single-blind controlled clinical trial we studied 45 healthy volunteers divided into three groups. Group 1 received d-α-tocopherol 1,200 I.U. daily; Group 2 ascorbic acid 2 g daily and Group 3 ascorbic acid 2 g plus d-α-tocopherol 1,200 I.U. daily. Treatment was sustained for one week. Before and after treatment, the minimal erythema dose was determined in all participants. The results show that the median minimal erythema dose increased from 60 to 65 mJ/cm^2 in Group 1 and from 50 to 70 mJ/cm^2 in Group 3. No modifications were observed in Group 2. We conclude that d-α-tocopherol prescribed in combination with ascorbic acid produces the best photoprotective effect. ***Key words: UV radiation absorption; vitamin C; vitamin E.***

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In the past few years, great interest has been taken in the participation of reactive oxygen species (ROS) in many biological processes and in the etiopathogenicity of diseases that affect several organs and systems. ROS encompass a variety of diverse chemical species, including superoxide anions, hydroxyl radicals and hydrogen peroxide. As is known, there is a normal production of ROS in the organism, which is checked and regulated by an antioxidant defence system, maintaining a balanced homeostasis. When this balance is lost, ROS may produce alterations in DNA, proteins, membrane phospholipids or in enzymes necessary for cellular homeostasis (1, 2).

The metabolism normally generates oxidant products, and external agents such as environmental toxins, inflammatory cytokines, chemotherapeutics and ultraviolet (UV) light promote their production (1). On the other hand, it is known that when there is sufficient UV radiation and it is absorbed by the skin, it is capable of inducing a biological response represented by oxidative stress and mediated by the formation of free radicals, ROS and lipid peroxidation with the subsequent formation of prostaglandins (PG). This photooxidative damage can cause the formation of erythema, premature aging of the skin, photodermatoses and skin cancer (3,4).

A previous study carried out on a human skin model has shown that UV irradiation provokes oxidative damage and increases the production of PGE2. A diminution of diverse cellular antioxidants and immediate oxidative damage were observed at elevated levels of UV irradiation (at least one dose of minimal erythema) (5).

The skin has a sophisticated antioxidant defence system which protects against excessive ROS production, among which the following stand out: 1) *enzymatic antioxidants*, superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase and catalase, 2) *lipophilic antioxidants*, α-tocopherol and ubiquinol, 3) *hydrophilic antioxidants*, ascorbic acid and uric acid, and other non-enzymatic models characterized by molecules of low molecular weight, such as pyruvate, flavonoids, carotenoids and glutathione. As is also known, these antioxidants are higher in the epidermis than in the dermis (6).

In recent years, it has been found that nitric oxide (NO), synthesized by keratinocytes, melanocytes and endothelial cells, acts as a potent inhibitor of lipid peroxidation (7, 8).

Based on the endogenous antioxidant system, the administration of antioxidants capable of scavenging ROS is considered to be a promissory strategy to reduce cutaneous reactions induced by the absorption of UV radiation. Among these antioxidants are vitamins E and C (9).

Vitamin E (d-α-tocopherol) is the main natural cutaneous non-enzymatic antioxidant soluble lipid protector

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against the effects of oxidative stress. Its antioxidant action possibly takes place through direct action on the singlet oxygen or superoxide anion, or through interruption of the chain reaction of free radical formation, by hydrogen donation to free radicals favouring the formation of low-energy compounds (10). It has been demonstrated that d-α-tocopherol also has photoprotective activity, and it should be mentioned that in most of these studies d-α-tocopherol was administered topically (11). In human beings, studies with oral administration of tocopherol have been limited, the administrated doses have been variable, and the results have not been uniform, although some results are encouraging (12–14).

Vitamin C (ascorbic acid) is an electron donor for inter- and extra-cellular chemical reactions. It reduces radical superoxide, hydroxyl (OH) and other reactive oxidants. Ascorbic acid acts as an antioxidant by scavenging free radicals and by regenerating vitamin E from its radical form. It may also act as a pro-oxidant in the presence of transition metal ions, such as iron. In human and animal models photoprotective action has been found with its topical application (11). This action has not been demonstrated by oral administration in humans, and has only been identified in the joint administration of ascorbic acid and d-α-tocopherol, probably through a synergistic mechanism (11, 13, 14).

From what has been previously mentioned about the photoprotective action of vitamins C and E, and the few clinical studies related with its administration in humans, we consider it to be of interest to carry out a clinical assay to determine whether the therapy of independent or combined administration of ascorbic acid and d-α-tocopherol has a photo-protective effect in healthy individuals, significantly enhancing the minimal erythema dose (MED).

MATERIALS AND METHODS

Design of the Study

We performed a single-blind controlled clinical assay with 45 healthy volunteers, with an average age of 28.6 ± 8.6 years (range 18–44 years); 29 women and 16 men. The dominant skin type was Type III, with 28 patients (62.2%), 13 subjects were Type IV (28.9%), and only 4 volunteers were Type II (8.8%). The study was approved by the local ethics committee of the Instituto Mexicane del Sequro Social.

The subjects had received no vitamin supplementation before the experiment, there were no photosensitivity antecedents, and written consent was obtained. An individual not directly involved in the study randomly assigned the 45 volunteers into three groups of 14, 15 and 16 individuals. Both treatment assignment and evaluation were blinded for the investigators up until the study's conclusion. The three groups received daily treatment in a single dose for one week: Group 1: 1,200 I.U. d-α-tocopherol, Group 2: 2 g ascorbic acid, and Group 3: 2 g ascorbic acid and 1,200 I.U. d-α-tocopherol. MED was determined in all participants before and after treatment by one individual who did not know the assigned treatment (blind) for each individual.

Determination of the Minimal Erythema Dose

A special photosensitivity test patch was placed on the paravertebral region (right and left, before and after treatment, respectively) of all participants (MED Tester). The UVB dose was increased by $10\,mJ/cm^2$ at a time from 30 to $140\,mJ/cm^2$. The phototherapy lamp (PH36, 800 Watts, Cooper Hewitt, KBD, Inc.) used in the experiment was previously calibrated with an Eppley radiometer model TUVR (total UV radiation) having a spectral interval of (290–360 nm). The radiation intensities obtained indicated a UVB irradiance flow from the PH36 at a distance of 36 cm of $0.302\,mW/cm^2$.

Statistics

The results were statistically analysed using methods of descriptive and inference statistics such as the Student t-test, the Wilcoxon test and the Kruskal-Wallis variance analysis contained in different statistical software packages as SPSS 8, GraphPAD in Stat and Primer of Biostatistics.

RESULTS

All volunteers completed the study: 14 in Group 1 (vitamin E), 15 in Group 2 (vitamin C) and 16 in Group 3 (vitamins C and E).

Using the Kruskal-Wallis test the following was observed for the basal determination: The median of MED did not show significant differences among the three treatment groups ($p = 0.4$). However, after the study, the determination showed a significant increase ($p = 0.04$).

Of the 14 patients included in Group 1 (d-α-tocopherol), 9 of them showed an increase in the threshold of MED; in 7 the increase was $10\,mJ/cm^2$ and in 2 it was $20\,mJ/cm^2$. In the last 5 volunteers there were no estimated changes with respect to the basal determination. There was a significant difference ($p = 0.002$) in the median comparison of the MED values of Group 1 before ($60\,mJ/cm^2$) and after ($65\,mJ/cm^2$) using the Wilcoxon test.

The median comparison of MED from Group 2 (ascorbic acid) did not show significant results with the Wilcoxon test ($p = 0.5$) (before treatment $60\,mJ/cm^2$ and after treatment $60\,mJ/cm^2$). Only one of the individuals showed an increase in MED.

In Group 3 (ascorbic acid plus d-α-tocopherol), 15 out of 16 patients showed an increase of MED. In 10 of them, the increase was $20\,mJ/cm^2$, in 4, $10\,mJ/cm^2$, and in 1 it reached $30\,mJ/cm^2$. The comparison of the median values of MED with the Wilcoxon test showed a significant difference ($p = 0.0001$) (before $50\,mJ/cm^2$ and after $70\,mJ/cm^2$).

Finally, a paired MED comparison was carried out by skin type, before and after each treatment. As shown in Table I, Groups 1 and 2 showed no significant difference among the three skin types, before and after treatment. However, Group 3 showed a significant improvement in the three skin types, especially in Types III and IV.

Table I. *Minimal erythema dose by skin type, before and after treatment*

Skin type	*Minimal erythema dose* mJ/cm^2 (mean ± SD)		
	Before	After	p*
Group 1			
II ($n = 1$)	40	40	–
III ($n = 9$)	56.6 ± 7	64.4 ± 12.3	(0.1)
IV ($n = 4$)	65.7 ± 5.7	75 ± 12.9	(0.1)
Group 2			
II ($n = 1$)	40	40	–
III ($n = 8$)	56.2 ± 10.5	56.2 ± 7.4	(0.9)
IV ($n = 6$)	70 ± 12	70 ± 12	(0.9)
Group 3			
II ($n = 2$)	40	60	–
III ($n = 11$)	56.3 ± 8	71.8 ± 7.5	(0.0005)
IV ($n = 3$)	60 ± 10	80 ± 10	(0.01)

*Student t-test paired data.

DISCUSSION

In our study, a statistically significant increase in MED was observed in the groups treated with 1,200 I.U. of d-α-tocopherol or with the combination of 2 g of ascorbic acid plus 1,200 I.U. of d-α-tocopherol, the increase being greater with the combined treatment. In clinical terms, the MED increase in the group treated with vitamin E was insignificant, with a median increase of only 5 mJ/cm^2, and the group treated with vitamin C as the only supplement showed no significant changes. However, in the group treated with both vitamins the median increase was 20 mJ/cm^2.

Our results concur with those of other authors in regard to the significant increase in the MED in subjects treated with a combination of ascorbic acid and d-α-tocopherol (14). These findings can be explained by the synergic action between both vitamins, which has been demonstrated in *in vitro* and *in vivo* studies. It is known that ascorbic acid regenerates the antioxidant form of vitamin E and that this interaction is present in lipid peroxidation inhibition (15–17).

Some of the increases obtained in MED by different authors who agree upon daily administered doses of α-tocopherol and ascorbic acid are reproduced here: Eberline-Konig et al. (13) obtained an increase in the MED median of 68.5 mJ/cm^2 to 96.5 mJ/cm^2 with 1,000 I.U. of tocopherol combined with 2 g of ascorbic acid; Fuchs & Kern (14) obtained a MED of 103 mJ/cm^2 to 183 mJ/cm^2 administering 2,000 I.U. and 3 g of vitamins E and C, respectively, while we obtained 50 mJ/cm^2 to 70 mJ/cm^2 with 1,200 I.U. of α-tocopherol and 2 g of ascorbic acid. It may be inferred that the photoprotective effect could be dosage-dependent; however, more studies are needed to prove this hypothesis. In clinical terms, we must consider that even though the doses used in the previous studies are considered safe (18), they may be excessive for some patients. Therefore we must question in which photodermatosis they should be used and for how long. Finally, in our study we found no significant differences in the comparison of MED among the skin types or among treatments; however, larger and more homogeneous samples need to be studied in order for this to be affirmed or denied.

From our findings, we conclude that the combined administration of both vitamins is required in order to confer an important clinical photoprotective effect, as described by other authors. The recognition of this photoprotective effect in vitamins C and E could favour their combined use as an auxiliary in UV radiation protection means in those individuals presenting with photosensitive dermatosis or in those in whom photoprotection with sunscreens is not well tolerated. Further studies are needed to find the precise optimum dose and to analyse its effect in individuals with photodermatosis, as until now this has only been demonstrated in clinically healthy subjects.

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