A Double-Blinded, Randomized, Controlled Trial to Quantitate Photoprotective Effects of an Antioxidant Combination Product

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ABSTRACT

Background: Ingestion of multiple antioxidants may result in synergistic increases in skin protection. **Methods:** In a double-blind, randomized, controlled study, the authors evaluated the effect of an antioxidant combination product in women with mild-to-moderate photoaging over 20 weeks. Changes on Oxygen Radical Absorbance Capacity levels and Minimal Erythema Dose were measured throughout the study. **Results:** Both Minimal Erythema Dose and Oxygen Radical Absorbance Capacity levels increased in women receiving the antioxidant combination product, with the difference from baseline being statistically significant as early as Week 4. Similar findings were observed in women who received the control product, which had modest antioxidant activity. The comparisons between the two groups were not statistically significant. **Conclusion:** Oral ingestion of a combination of antioxidants can lead to improvement on objective measurements, such as Minimal Erythema Dose and Oxygen Radical Absorbance Capacity levels, when compared to baseline values. (*J Clin Aesthet Dermatol.* 2012;5(4):29–32.)

Aging of the skin is caused by intrinsic mechanisms that are primarily genetically determined and by extrinsic ones that depend on environmental factors, including ultraviolet (UV) radiation exposure, especially through generation of reactive oxygen species (ROS).[1] Photoprotection, through the use of ingestible antioxidant substances, has been shown to ameliorate the damaging effects of UV radiation.[2,3] Some of these substances increase the protective barrier against UV radiation through UV absorbing activity, protection against cellular damage from ROS through antioxidant effects, and suppression of enzymes that degrade skin structure, such as matrix metalloproteinases (MMP). There is increasing evidence that combinations of antioxidants may work synergistically.[4,5] Therefore, a mixture of antioxidants could be optimized in order to prevent photodamage. In addition, the development of natural strategies to address the effects of UV radiation on the skin is highly desirable. An equilibrium between the different antioxidants in the skin could be optimized in order to prevent photodamage.[6]

There have been few studies addressing objective effects of combination antioxidant products on measurements, such as Oxygen Radical Absorbance Capacity (ORAC) levels and Minimal Erythema Dose (MED). Here, the authors present objective measurements from a larger study on the effects of an antioxidant combination product (ACP) designed at evidence-based potencies to optimize effectiveness tested for protection of the skin against UV radiation damage and improvement of the appearance of the skin.

PATIENTS AND METHODS

Participants. Fifty-six women with mild-to-moderate photoaging, graded as types II or III on the Glogau Photoaging Classification[7] at their baseline visit, between the

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TABLE 1. Demographic and clinical characteristics at baseline

	ANTIOXIDANT COMBINATION PRODUCT GROUP	CONTROL GROUP
AGE, YEAR, MEAN (SD)	49.1 (6.9)	50.6 (7.3)
REPRODUCTIVE STATUS, POSTMENOPAUSAL, NO. (%)	13 (46.4)	17 (60.7)
RACE, CAUCASIAN, NO. (%)	24 (85.7)	26 (92.9)
MED, mJ/cm^2, MEAN (SD)	64.7 (16.0)	62.6 (19.4)
ORAC, UNITS, MEAN (SD)	9064 (2734)	8705 (2016)

MED=Minimal Erythema Dose; ORAC=Oxygen Radical Absorbance Capacity. No statistically significant differences were noted between groups

ages of 35 and 62 who are nonsmokers, have a body mass index (BMI) lower than $30kg/m^2$, and Fitzpatrick skin types from I to IV were enrolled in this study from January to March 2008. Subjects using systemic or topical retinoids or steroids, topical alpha-hydroxy acids, and antioxidants (e.g., green tea, Vitamin C, and Vitamin E) at Baseline were excluded. Use of sunscreen was strongly recommended during the study.

Study design. This 20-week, prospective, controlled, double-blinded, randomized study was approved by the institutional review board of the Massachusetts General Hospital and was conducted in compliance with the Declaration of Helsinki guidelines. Eligible subjects who provided written informed consent were randomized 1:1 to take the ACP or control product (28 subjects in each group) daily. The active ingredients of the ACP include vitamin A, vitamin C, vitamin E, selenium, pomegranate extract, quercetin, green tea extract, coenzyme Q10, mixed tocopherols, epigallocatechin-3-gallate (EGCG), lutein, lycopene, and zeaxanthin. The control product had approximately 15 percent of the antioxidant activity levels of the ACP. A food frequency questionnaire was given at baseline in order to evaluate any potential differences in dietary habits between the two groups.

Clinical assessments. Changes in plasma antioxidant capacity (AOC) were measured by the ORAC assay.[8] ORAC levels were determined at Baseline and Week 4. MED test was performed using a UVB light source (Panosol UVB®, National Biological Corporation, Twinsburg, Ohio) at Baseline and Weeks 4, 12, and 20. Six 2x2cm squares were exposed to different doses of UVB depending on the Fitzpatrick skin type at $10mJ/cm^2$ intervals. MED was defined by minimum dose of UVB necessary to generate erythema 24 hours after the exposure.

Statistical analysis. MED at 4, 12, and 20 weeks and ORAC levels at four weeks were secondary efficacy endpoints. Statistical calculations were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina). For the continuous data, two-tailed, independent, and paired t-tests were performed with p values noted. For the categorical data, Fisher's exact test was performed. Comparisons were performed using an intent-to-treat analysis that included all randomized subjects. Statistical significance was determined at $p<0.05$.

RESULTS

Most subjects finished the study, with a total of eight subjects (14%) withdrawing from the study before completing 20 weeks. The treatment groups were well balanced in terms of demographic and baseline clinical data (Table 1). The mean age was about 50 years and the majority of the subjects were fair skinned. The proportion of postmenopausal women was slightly, but not significantly, higher in the control group versus the ACP one (p=0.40). There were no statistically significant differences in the MED and ORAC levels between the groups at Baseline nor were there significant differences in self-reported fruit and vegetable intake between the two groups at Baseline.

MED increased progressively in the ACP group, with the difference from baseline being statistically significant as soon as Week 4. The comparisons between the two groups were found not to be statistically significant. The same trend was observed for ORAC levels, where the ACP group had significantly increased levels at Week 4 (p=0.01). Similar findings were observed in the control group (Table 2).

After 20 weeks, some clinical parameters of aging improved significantly in both physician's and subject's opinion. These global assessments were not shown here because of their subjectivity and potential impreciseness.

Withdrawals due to adverse events occurred similarly in both groups. Three subjects in the ACP group experienced nausea and/or bloating after consuming the product. This led to one subject discontinuing the study. There were no serious adverse events in either group.

DISCUSSION

The ACP group in this study experienced improvement of clinical and laboratory parameters related to oral ingestion of antioxidants as evidenced by MED and ORAC results. Interestingly, there was a lack of correlation between the antioxidant levels (ORAC) and the MED results. This may be explained by the fact that the ACP contains 19 different ingredients and the antioxidant activity of these ingredients that could be detected in the ORAC levels may not be the

TABLE 2. Mean (± SD) for ORAC and MED by randomized group

	ANTIOXIDANT COMBINATION PRODUCT (N=24)	CONTROL (N=25)	*P* VALUE§
ORAC BASELINE	9064 ± 2734	8705 ± 2016	
Week 4 Difference between Baseline and Week 4 *P* value*	10683 ± 1983 1619 ± 2851 0.01	9792 ± 1489 1088 ± 1965 0.04	0.45
MED BASELINE	64.7 ± 16.0	62.6 ± 19.4	
Week 4 Difference between Baseline and Week 4 *P* value*	73.3 ± 18.2 8.6 ± 17.7 0.02	69.8 ± 14.5 7.2 ± 16.3 0.03	0.75
Week 12 Difference between Baseline and Week 12 *P* value*	75.4 ± 21.2 10.7 ± 18.2 0.004	73.2 ± 16.0 10.6 ± 22.3 0.02	0.98
Week 20 Difference between Baseline and Week 20 *P* value*	78.2 ± 22.6 13.5 ± 21.1 0.002	73.2 ± 22.5 10.6 ± 21.3 0.02	0.62

MED=Minimal Erythema Dose; ORAC=Oxygen Radical Absorbance Capacity
* Paired *t*-test for within group comparison of 4, 12, and 20 weeks to baseline
§ Two-sided *t*-test for the differences in ORAC and MED levels between the two groups

same ones responsible for the changes in MED levels. Further research about what these values mean are likely needed.

In this study, the ACP and control products led to an increase of 17.9 and 12.5 percent in ORAC units after four weeks, respectively. These increments were higher than those observed in other studies evaluating the effect of other antioxidant combinations. This could have resulted from a synergistic effect. Fish oil, containing a natural carotenoid mixture (β-carotene, α-carotene, lycopene, bixin, lutein, and paprika carotenoids) led to increased plasma ORAC levels by 4.5 percent after three weeks.[9] Huang et al[10] evaluated the effect of vitamin C and vitamin E alone or combined versus placebo. They found that only vitamin C led to an increase of serum ORAC levels after two months (2.7%). The levels decreased over time in the placebo (3.0%), vitamin E (1.9%), and vitamin C plus E (0.4%) groups.[10]

In addition, the authors present selected studies evaluating the effect of oral antioxidants on MED. A study that evaluated 16 patients either taking supplements containing vitamin E or β-carotene for eight weeks showed no change in median MED in both groups.[11] A randomized, controlled study comparing the effect of lycopene in olive oil versus olive oil alone did not demonstrate a statistically significant difference in MED between the two groups after 12 weeks.[12] Other studies evaluating selected doses of antioxidant supplements, such as β-carotene, lycopene, and α-tocopherol, showed an increased MED.[13]

There was a progressive improvement in MED levels in the ACP group over time until 20 weeks. These levels also increased in the control group, but they reached a plateau at 12 weeks. It is possible that, if the trial had continued and this pattern remained constant, the differences between the groups would become statistically significant.

This study presents some limitations. The study was likely underpowered to detect statistically significant differences between the two products, and the control product did not serve as a true control due to its antioxidant activity. In order to make the two beverages more similar in appearance and taste, the authors could not avoid including some flavorings that had antioxidant activity, which were more potent than anticipated in terms of their effects on ORAC levels. Another possibility for the non-significant differences between the two groups is that the duration of the study was not long enough to detect significant differences between the two groups. Despite these limitations, the ACP had positive clinical effects. Further study of antioxidants and MED are warranted to determine the mechanism of action. In addition, more research is needed to understand the biological effects of blood ORAC levels.

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