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Dichotomous attributes of BHT, vitamin E and vitamin C as antioxidant and anti-glycation molecules in skin models

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INTRODUCTION

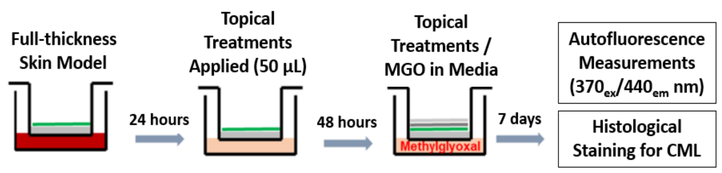
Antioxidants have versatile applications in skincare and dermatology, ranging from the neutralization of reactive oxygen species (ROS) to more indirect effects on cellular enzymes and macromolecular structures such as cell nuclei and elastic fibers in the skin. Oxidative stress and damage to these structures can also result from biochemical reactions such as glycation, which are linked to aging. Several biological mechanisms are known to prevent the formation of advanced glycation end products (AGEs), one of which is the inhibition of ROS formation.[1,2] Here, we performed an analysis of the antioxidant capacities of three common antioxidants, butylated hydroxytoluene (BHT), Vitamin E, and Vitamin C, to determine their effects on the accumulation of oxidative stress and AGEs in the dermis of 3D skin models cultured in the presence of glycation agent methylglyoxal (MGO). AGEs were detected in skin models using measurements of skin autofluorescence[3–4] and histological staining of the AGE carboxmethyllysine. Although all three antioxidants had a significant capacity to inhibit the formation of AGEs, there were notable differences in the concentrations corresponding to the degree of protection against glycation-induced damage. The results of this study show that topical treatment with antioxidants can inhibit glycation in skin models and that the synergism between modulation of oxidative stress and glycation prevention is dependent on the specific concentration and antioxidant power of the individual molecules.

METHODS

Total Antioxidant Capacity

Total antioxidant capacity of 0.1%, 0.2%, and 0.5% of three antioxidant solutions was based on the conversion of Cu^{2+} to Cu^{+} in an OD 570 nm colorimetric assay and expressed relative to Trolox power as a standard.

Treatment of Skin Models



- Full-thickness skin models were equilibrated for 24 h in culture media at 37˚C, 5% CO_2/95% humidity.
- After 24 h, 50 µL topical application of each treatment. Untreated (UT) control, no topical application.
- After 48 h, basal media was replaced with media plus 500 µM MGO, to induce glycation; topical treatments were replaced.
- Basal media with MGO and topical treatments were changed every 48 h over 7 days.

Treatment	Topical (50 µL)	Basal (5 mL) 500 µM MGO
UT Ctr	—	No MGO
UT MGO Ctr	—	✓
Vehicle Ctr	PBS	✓
Vitamin C	0.1%, 0.2%, 0.5%	✓
Vitamin E	0.1%, 0.2%, 0.5%	✓
BHT	0.1%, 0.2%, 0.5%	✓

Autofluorescence Measurements for Glycation

Autofluorescence ($370_{ex}/440_{em}$ nm) of skins was measured with a microplate reader on days 0, 1, 4, and 7.

Histological Staining for Carboxymethyllysine (CML)

On day 7, 5-µm sections were stained with CML antibody. Fluorescence signal intensities were measured from micrographs in ImageJ. Relative signal intensity was normalized to the untreated control on day 0.

RESULTS

1) Total Antioxidant Capacity

For total antioxidant capacity, BHT and Vitamin E show linear dose responses; whereas Vitamin C shows an inverse dose response.

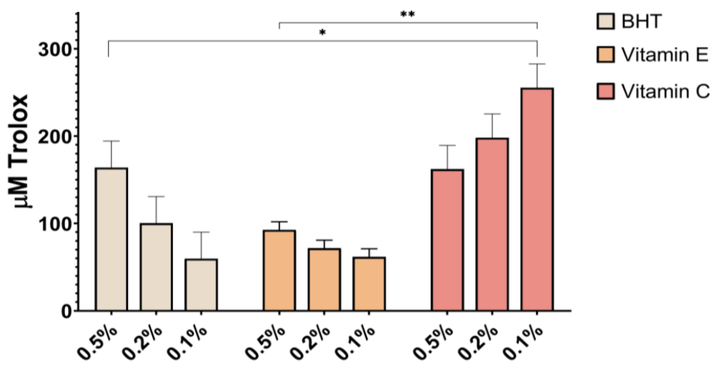


Figure 1. Total antioxidant capacity of BHT, Vitamin E, and Vitamin C based on conversion of Cu2+ to Cu+ in a colorimetric assay; expressed relative to Trolox power.

2) Antioxidants Prevent Glycation in Skin Models

All three antioxidants significantly inhibited formation of glycation products; however, there were differences in concentrations corresponding to the degree of protection against glycation-induced damage.

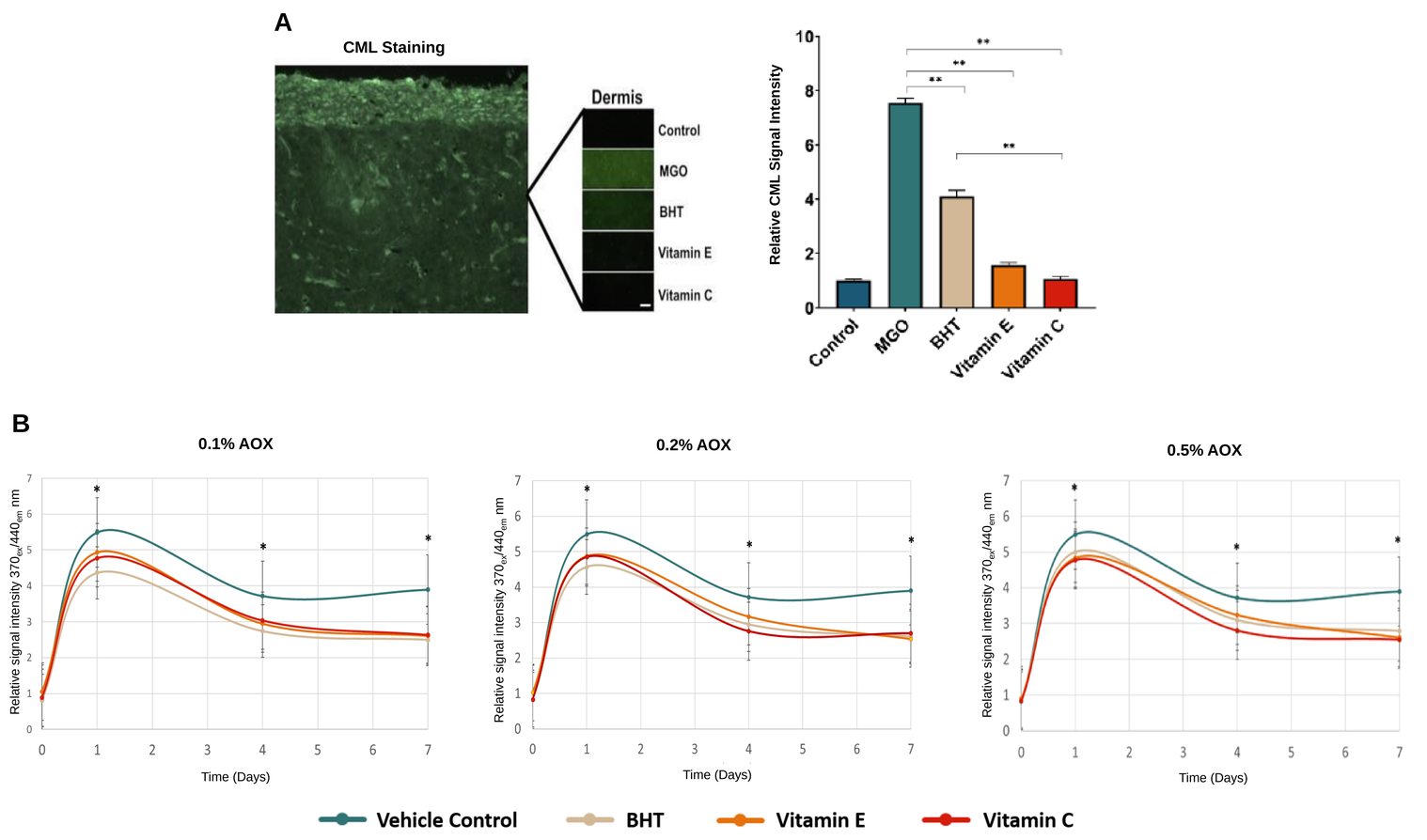


Figure 2. Anti-glycation effects of BHT, Vitamin E, and Vitamin C in 3D full-thickness skin models. Skin models were cultured in the presence of MGO and treated topically with 0%, 0.1%, 0.2%, and 0.5% BHT, Vitamin E, and Vitamin C for 7 days. (A) CML antibody staining of skin models treated with 0.5% antioxidants. (B) Glycation of skin models measured by autofluorescence ($370_{ex}/440_{em}$ nm).

CONCLUSIONS

- ❑ Topical treatment with antioxidants can inhibit glycation in skin models.
- ❑ Synergy between modulation of oxidative stress and glycation prevention is dependent on the specific concentration and antioxidant power of the individual molecules.
- ❑ This detailed understanding can inform fine-tuned approaches to formulating with active ingredients tailored to personalized skincare.

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