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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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Purpose

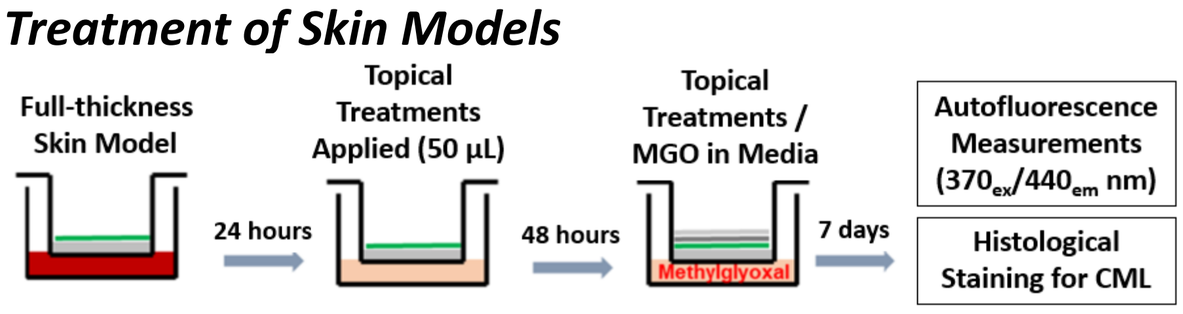
Antioxidants have versatile applications in skincare and dermatology, ranging from the neutralization of reactive oxygen species to more indirect effects on cellular enzymes and macromolecular structures such as cell nuclei and elastic fibers in the skin. Oxidative stress and damage to these structures can also result from biochemical reactions such as glycation, which are linked to aging. 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 advanced glycation end products (AGEs) in the dermis of 3D skin models cultured in the presence of glycation agent methylglyoxal (MGO).

Methods

Total Antioxidant Capacity

Total antioxidant capacities 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

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

Total Antioxidant Capacity

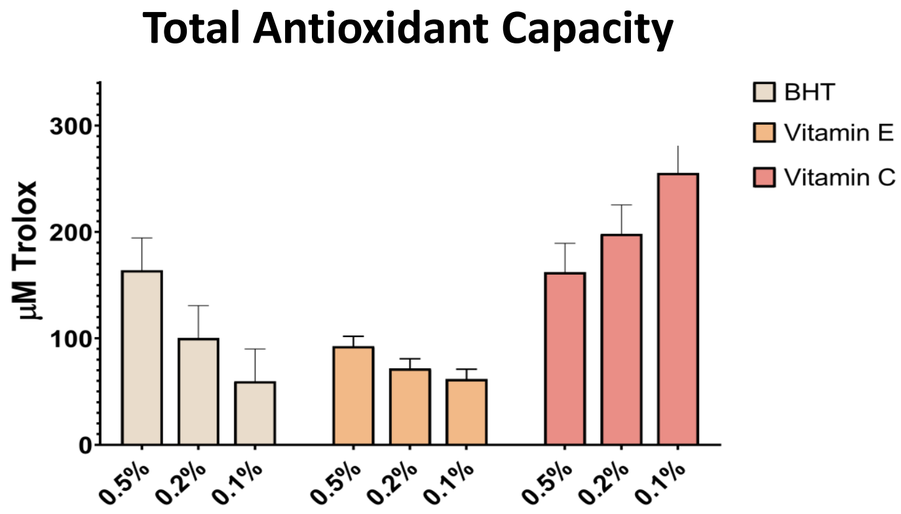
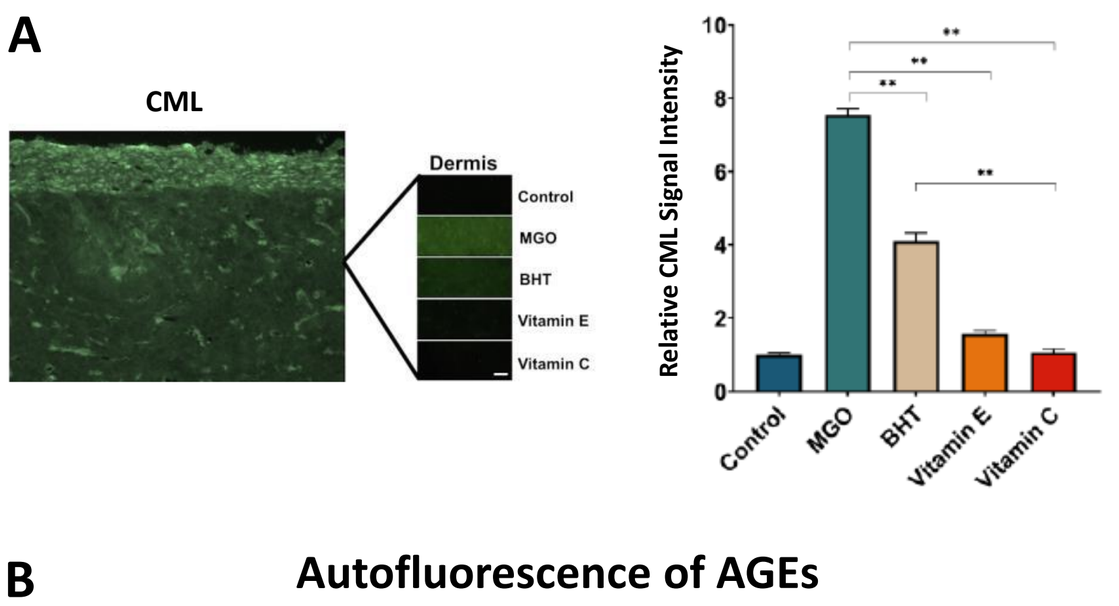


Figure 1. Total antioxidant capacity of BHT, Vitamin E, and Vitamin C based on conversion of Cu^{2+} to Cu^{+} in colorimetric assay; expressed relative to Trolox power.



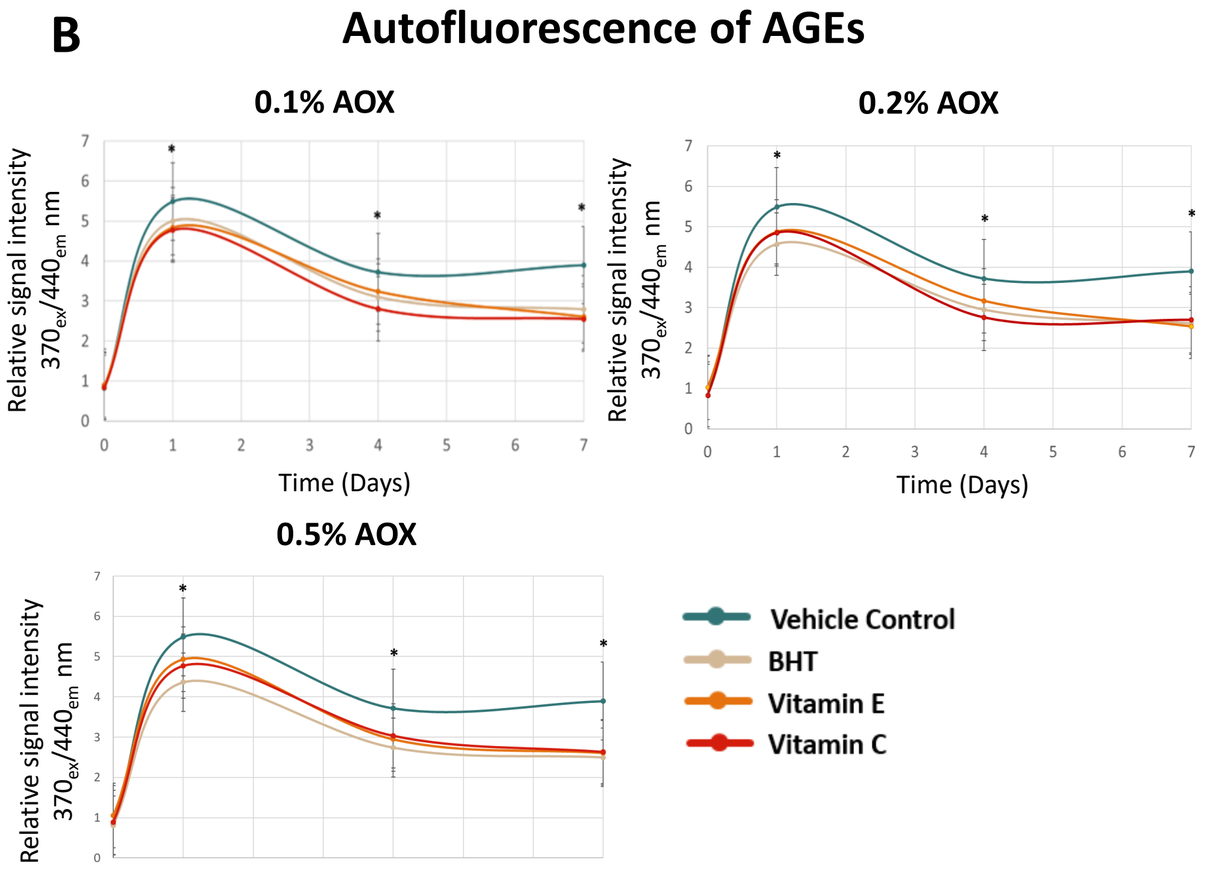


Figure 2. Anti-glycation effects of BHT, Vitamin E, and Vitamin C in 3D full-thickness skin models. Skin models were cultured in the presence of MGO and treated topically with 0%, 0.1%, 0.2%, and 0.5% BHT, Vitamin E, and Vitamin C for 7 days. (*A*) CML antibody staining of skin models treated with 0.5% 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 can inform fine-tuned approaches to formulating with active ingredients tailored to personalized skincare.