



A Topical Antioxidant Solution Containing Vitamins C and E with Ferulic Acid Protects Human Skin from Sunlight Damage and DNA Mutations Associated with Skin Cancer



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Background

Sunlight exposure generates oxidative stress in skin that can result in photodamage, including photoaging and skin cancer. The body uses antioxidants to neutralize oxidation of nucleic acids, proteins and lipids; but protection achieved by oral ingestion is limited (Placzek et al., 2005). To augment antioxidant protection, we have developed and reported three generations of topical antioxidant formulations, each with progressively better protection against solar-simulated radiation. The current formulation contains physiologic antioxidants, vitamins C and E, stabilized by a potent plant antioxidant, ferulic acid; optimized for chemical stability, percutaneous absorption, and photoprotection (Lin et al., 2005).

Methods

In this study, which was conducted at Duke University Medical Center, with the approval of the Duke Institutional Review Board, two different solutions were applied ($2\text{mg}/\text{cm}^2$) to back skin of human volunteers daily for four days. One solution was an aqueous solution containing 15% L-ascorbic acid, 1% dl-tocopherol and 0.5% trans ferulic acid (C E Ferulic); the other, a vehicle control solution. On day four, solar-simulated ultraviolet radiation, passed through a WG295 Schott selective UVB band-high pass filter to eliminate wavelengths less than 295 nm was administered to each patch of treated skin. The vehicle patch received 2-6x MED and the C E Ferulic patch received 2-10x MED of solar-simulated irradiation, each at 2x MED intervals. One day later, skin was evaluated by colorimeter for erythema and biopsies of 6x MED-irradiated skin were evaluated for sunburn cells. Erythema was measured by computerized colorimetry evaluation in the "a" mode of digital skin photographs (Tournas, 2005). Each spot and adjacent unirradiated skin was measured in triplicate. The difference between irradiated and unirradiated skin determined the erythema. Sunburn cells were determined in formalin-fixed 8mm punch biopsy sections stained with hematoxylin and eosin. The p values were calculated by Student test. An additional four subjects who received 1-5x MED of solar-simulated irradiation were biopsied for immunohistochemistry of thymine dimers and p53.

Results

C E Ferulic provided substantial protection against erythema (Fig 1, 2). At 2-6x MED irradiance, colorimeter readings for vehicle vs. C E Ferulic revealed significant ($p < .01$) photoprotection by C E Ferulic. Moreover, C E Ferulic provided significant ($p < .01$) protection at 8x MED and 10x MED when compared to 6x MED irradiated vehicle-treated skin. Also, sunburn cell enumeration of 6x MED-irradiated skin (Fig 3) revealed significant ($p < .01$) protection by C E Ferulic, (vehicle 31.5 ± 14.3 vs C E Ferulic 8.4 ± 7) Immunohistochemistry of skin receiving 2x MED revealed virtually complete protection by C E Ferulic against UV-generation of thymine dimers (Fig 4) and p53 (Fig 5).

Discussion

This study demonstrates that a combination of physiologic antioxidant vitamins C and E, stabilized by a powerful plant antioxidant, ferulic acid, can be applied topically to skin and supplement the skin's own antioxidant pool to protect against UV-induced oxidative damage. In addition to protecting the skin against erythema and apoptosis associated with cellular damage, protection was also provided against UV-induced DNA mutations that have been demonstrated to be associated with skin cancer. It is unclear why antioxidants should affect thymine dimer formation. A recent laser-capture study of squamous cell carcinoma of skin and actinic keratoses revealed abundant thymine dimer mutations throughout the tumors (Agar et al., 2004; Halliday et al., 2005). Although generation of thymine dimer mutations has been considered to be caused by direct UVB absorption and unrelated to oxidation, recent observations have revealed abundant thymine dimer generation in skin by UVA irradiation (Kappes et al., 2006; Mouret et al., 2006), even though UVA is only minimally absorbed by DNA (Douki et al., 2003). UVA preferentially generates oxidative stress in skin in comparison to UVB (Hanson and Simon, 1998). Antioxidants have recently been demonstrated to effectively inhibit UVB-induced cyclopurimidine dimers in human HaCaT cells but not in naked DNA suggesting a cellular intermediate whose oxidation may be involved in cyclopurimidine dimer formation (Hochberg et al., 2006). In addition, UVA may

inhibit DNA repair enzymes allowing thymine dimers to persist unrepaired and enter into DNA replication (Courdavault et al., 2004). p53 is induced by UV-irradiation in response to DNA damage (Chao et al., 2000) and oxidative stress (Meplan et al., 2000). p53 causes the cell to slow DNA replication and subsequent cell division allowing the cell more time to repair DNA damage. Reduction of p53 induction by C E Ferulic in this study may relate to protection of DNA damage and reduction of oxidative stress afforded by the antioxidants.

Conclusion

Topical C E Ferulic provided substantial protection for human skin against solar simulator-induced oxidative skin damage, including erythema, sunburn cell formation and DNA mutations related to skin cancer.

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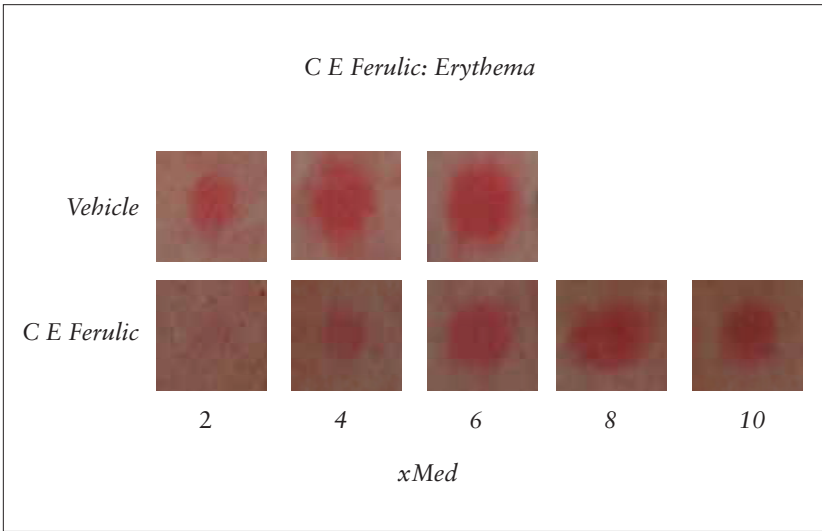


Figure 1
 C E Ferulic and Vehicle were applied to back skin (2mg/cm²) daily for 4 days. Skin was irradiated with solar-simulated ultraviolet radiation, 2x to 10x minimal erythema doses (MED) at 2x MED intervals. Erythema was determined one day later.

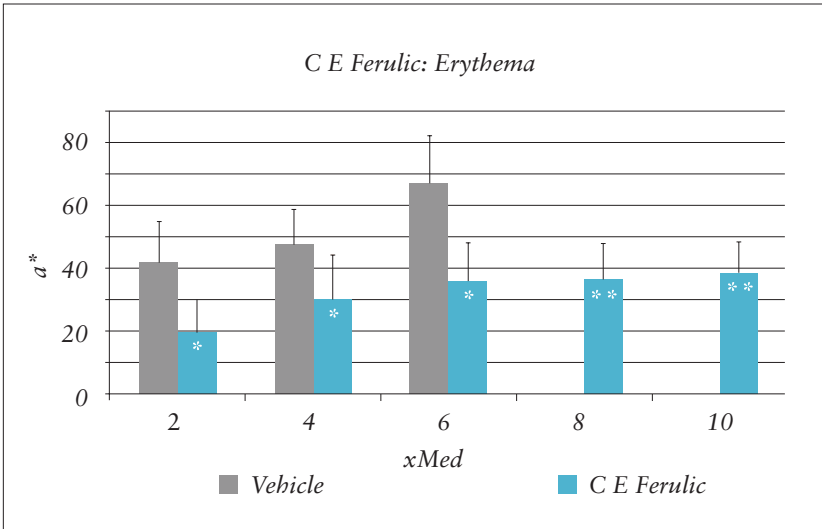


Figure 2
 C E Ferulic and Vehicle were applied to back skin (2mg/cm²) daily for 4 days. Skin was irradiated with solar-simulated ultraviolet radiation, 2x to 10x minimal erythema doses (MED) at 2x MED intervals. Erythema was determined one day later by colorimetry of digital photographs. Mean ± SD (n=9). * p<.01 vs vehicle. ** p<.01 vs vehicle at 6x MED.

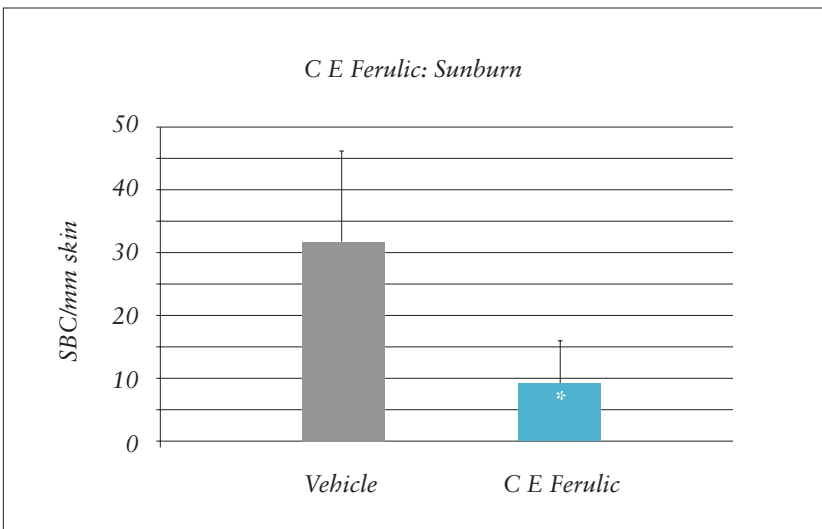
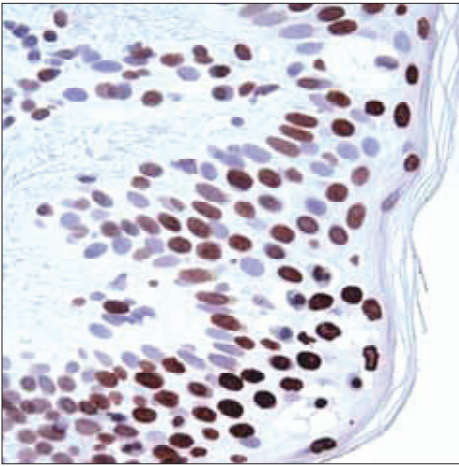
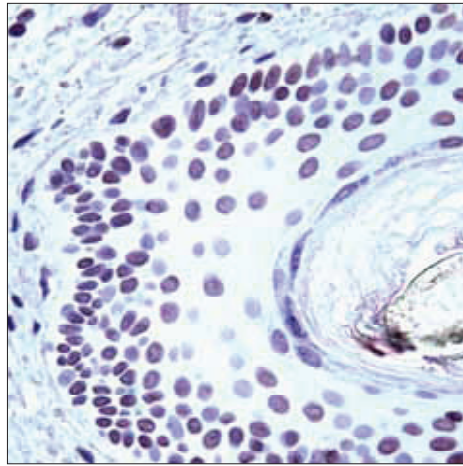


Figure 3
 C E Ferulic and Vehicle were applied to back skin (2mg/cm²) daily for 4 days. Skin was irradiated with solar-simulated ultraviolet radiation, 2x to 10x minimal erythema doses (MED) at 2x MED intervals. Skin biopsies of 6x MED-treated skin were taken one day later. Sunburn cells were counted and are expressed as cells per millimeter of epidermis. Mean ± SD (n=9). * p<.01 vs vehicle.

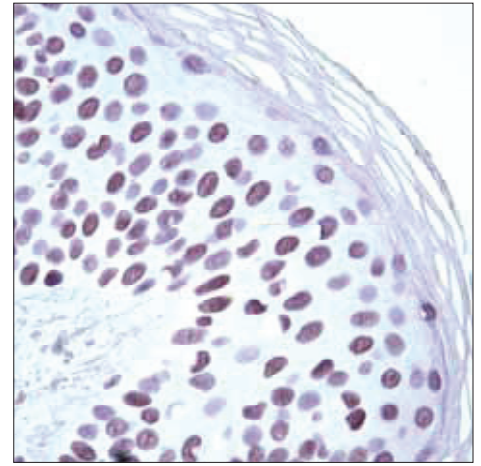
C E Ferulic: Thymine Dimers



Vehicle



C E Ferulic

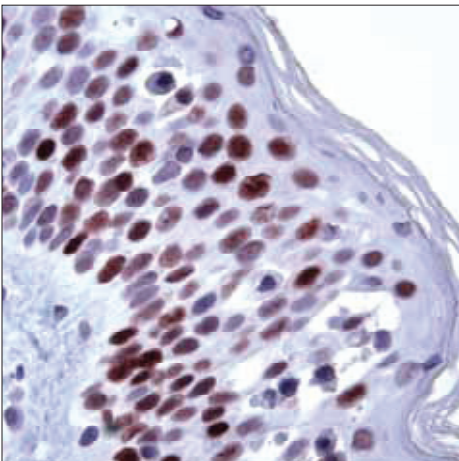


Unirradiated

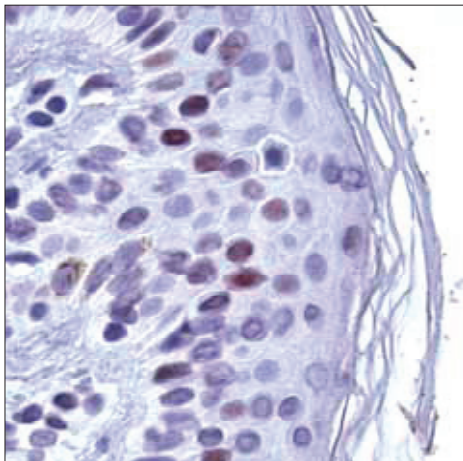
Figure 4

C E Ferulic and Vehicle were applied to back skin (2mg/cm²) daily for 4 days. Skin was irradiated with solar-simulated ultraviolet radiation, 1x to 5x minimal erythema doses (MED) at 1x MED intervals. Skin biopsies of 2x MED-treated skin were taken one day later and formalin-fixed tissue was stained for immunofluorescence using a mouse monoclonal antibody to thymine dimers (clone KTM53 Kamiya Biomedical Company). UV-generation of cellular thymine dimers was almost completely protected by C E Ferulic application.

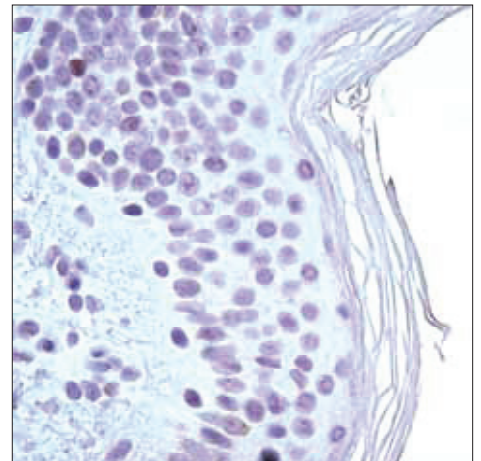
C E Ferulic: p53



Vehicle



C E Ferulic



Unirradiated

Figure 5

C E Ferulic and Vehicle were applied to back skin (2mg/cm²) daily for 4 days. Skin was irradiated with solar-simulated ultraviolet radiation, 1x to 5x minimal erythema doses (MED) at 1x MED intervals. Skin biopsies of 2x MED-treated skin were taken one day later and formalin-fixed tissue was stained for immunofluorescence using a monoclonal mouse antibody to human p53 (clone DO-7, DakoCytomation). UV-generation of p53 was almost completely protected by C E Ferulic application.