

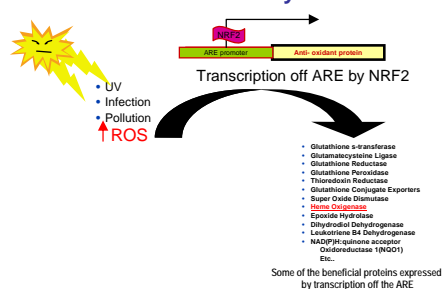
Skin Biomarkers Confirm the Anti-oxidant Activity of Olive Derivatives and Yeast Ferment Filtrate

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BACKGROUND

Reactive oxygen species (ROS) play a critical role in the process of UV-induced skin damage, skin photo-aging and melanogenesis. A family of enzymes and anti-oxidant proteins under control of the antioxidant response element (ARE) provide ideal biomarkers to monitor anti-oxidant activities in skin (figure below). The ARE family of proteins can protect against oxidative damage to cells not only by increasing endogenous antioxidant levels in cells, but also by up-regulating proteins that monitor for and repair the damage caused by ROS.

Up-regulation of Phase II Antioxidant Enzymes



OBJECTIVE

Determine the anti-oxidant effects of topically applied olive derivatives and yeast ferment filtrate (YFF) on anti-oxidant biomarkers, via transcription off the ARE and expression of related proteins.

METHODS

In vitro assays were performed using primary cultures of human skin keratinocytes and fibroblasts, and *ex vivo* human skin cultures, treated for 24 hours with olive derivatives (olive oil-derived fatty acids modified with PEG-7 (Olive Oil DFAP), olive oil blended with jojoba oil (Olive Oil BJO) and YFF.

ARE was assayed using the ARE-32 reporter cell line (CXR-Biosciences), a stable MCF7 reporter cell line containing 8 copies of the rat GST ARE linked to the Luciferase gene, which gives a robust luminescent light response to transcription off the ARE promoter.

Cell type and tissue confirmation of ARE were confirmed via an HO-1 ELISA assay, normalized to total protein per sample.

Statistical analysis was conducted using the Students t-test, comparing treated to control samples. Error bars indicate the SD.

RESULTS

Figure 1. ARE Activation Assay: Up-regulation of NRF2 transcription. Olive Oil DFAP (A), Olive Oil (B), and Yeast Ferment Filtrate (C) each produced significant dose-dependent up-regulation of NRF2 transcription in the ARE-32 reporter cell line. * $p < 0.02$

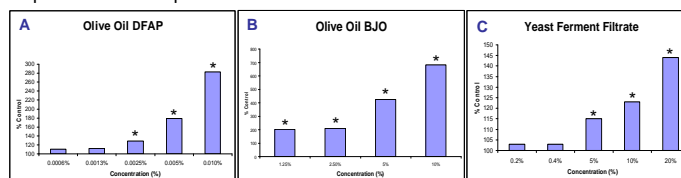


Figure 2. HO-1 in Human Skin Cells. Olive Oil DFAP, Olive Oil BJO and Yeast Ferment Filtrate produced significant dose-dependent increases in HO-1 expression, a biomarker of cellular antioxidant capacity, in human skin keratinocytes (A, C) and fibroblasts (B, D). * $p < 0.02$

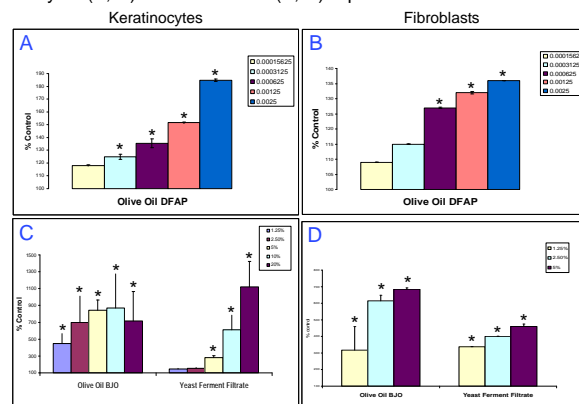


Figure 3. HO-1 in Human Skin Explants. Olive Oil BJO, Yeast Ferment Filtrate, and a YFF/Olive DFAP combination applied topically to human skin *ex vivo* produced increased expression of HO-1. p values for each treatment vs control are indicated.

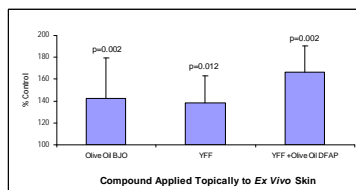
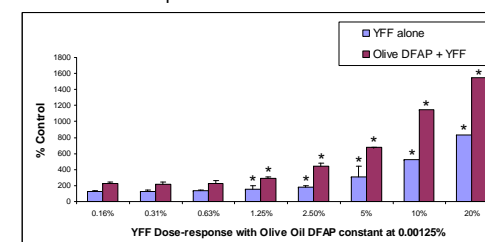


Figure 4. Synergistic increase in HO-1 in Human Skin Keratinocytes with Yeast Ferment Filtrate and Olive Oil DFAP. Olive Oil DFAP (at a low concentration that did not increase HO-1) was combined with increasing concentrations of YFF. * $p < 0.02$



SUMMARY

The anti-oxidant properties of the olive oil derivatives, Olive Oil DFAP and BJO, and Yeast Ferment Filtrate were confirmed by:

- Demonstration of transcription off the ARE in a reporter cell line (Figure 1).
- Increased HO-1 expression in primary cultures of human skin keratinocytes and fibroblasts (Figure 2).
- Increased HO-1 in human skin explant cultures (Figure 3).

Interestingly, ARE transcription was less affected by YFF than would be expected from HO-1 expression, suggesting an alternate pathway such as HIF-1 may be involved. Human skin keratinocytes treated with a level of Olive Oil DFAP too low to affect ARE on its own acted synergistically with YFF to increase HO-1 (Figure 4), indicating a synergistic anti-oxidant effect as well as supporting independent pathways.

CONCLUSIONS

These results support that the anti-oxidant properties of olive derivatives and Yeast Ferment Filtrate can provide powerful protection and repair for our skin against the continual assault of UV and environmentally induced ROS, and provide a compelling reason to further understand the complementary nature of these materials.