ABSTRACT

Human cells possess protection and repair mechanisms that may be described as "aging defense mechanisms." Nutrients are important cofactors for cellular function and protection; similarly, natural ingredients, including phytochemicals, have been shown to support key ADMs. The purpose of this study was to determine the effects of a novel nutritional supplement—NNS (containing fish oil, vitamins E and D, alpha-lipoic acid, Co-enzyme Q10, quercetin, citrus bioflavonoids—naringin and hesperidin, d-limonene, resveratrol, carotenoids—lycopene, lutein, and astaxanthin, purple corn and rosemary extracts) on ADMs (i.e. cellular protection). Forty healthy, non-smoking men and women between the ages of 40 and 75 years of age with Fitzpatrick skin types I and II were recruited to participate. Cellular injury was induced by 1% sodium metabisulphite solution (40 μM for 5 min) and 300 nm simulated ultraviolet radiation (UVR) exposure to non-irradiated skin. Assessments at baseline and 8 weeks post-supplementation included: skin erythema at all three MED doses and biopsy of the 3MED site to determine if apoptosis cells were determined. In addition, skin carotenoids levels were assessed non-invasively using Raman spectroscopy (Biophotonic Scanner, Nu Skin Enterprises, Provo, Utah). Was there was a significant effect of the NNS on ADMs following 8 weeks supplementation. Decrease in skin erythema at all 3 MED doses: 3MED (p=0.091), 2MED (p=0.011), and 1MED (p=0.001) were observed. Supplementation with the NNS led to a significant reduction in the mean number of apoptotic cells, 11.6 baseline vs. 5.7 cells/mm² at the 3MED dose at week 8, suggesting that the NNS protected against both UVR-induced DNA damage and apoptosis. Finally, skin carotenoids levels increased from 28600 Kaman Intensity Units (KIU) to 38775 KIU (p=0.001) indicative of an increase in antioxidant protection in the skin tissue post-supplementation. In conclusion, 8 weeks supplementation with the NNS supported key ADMs related to cellular health and function including protection against UVR cellular damage, apoptosis, inflammation and erythema.

INTRODUCTION

The human body has many mechanisms to respond to or limit the damage induced from environmental stressors. Many times the ability to repair or internal protective mechanisms are not sufficient to combat the insults and aging occurs. These insults can be generated both internally and externally and are defined as "aging aggressors." The mechanisms by which the body, tissues, or cells deal with these aggressors are described as "aging defense mechanisms" (ADMs).

EXAMPLES OF AGING DEFENSE MECHANISMS:

- Antioxidants and Protection Mechanisms
- Detoxification and Stress Response Mechanisms
- DNA Protection and Repair Mechanisms
- Tissue Renewal Mechanisms
- Mechanisms Regulating Metabolism
- Mechanisms Regulating Inflammatory Balance

One tissue that is particularly susceptible to environmental insults is the skin; furthermore it is an easily accessible tissue making it ideal to examine mechanisms of protection against aging.

Exposure of the skin to ultraviolet radiation (UVR) induces acute inflammation and characterized clinically by erythema or redness; furthermore, damaging UVR can also cause apoptosis of skin cells. Several studies have confirmed that acute exposure of human skin to UVR leads to oxidation of cellular biomolecules and to depletion of endogenous antioxidants. Skin UVR model provides a means to evaluate nutritional ingredients and protective effect of aging defense mechanisms like antioxidant protection, cellular stress response, and inflammatory balance.

We formulated a novel nutritional supplement (NNS) of natural ingredients to support several ADMs: Fish oil (Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)), resveratrol, quercetin, purple corn extract, rosemary leaf extract, citrus bioflavonoids, Coenzyme Q10, alpha-lipoic acid, astaxanthin, lycopene, lutein, vitamin D3, vitamin K1 (as menaquinone-7), and d-limonene. In this study we determined the impact of the NNS on ADMs associated with antioxidant and DNA protection, cellular stress response, and inflammatory balance mechanisms in response to damaging UVR.

OBJECTIVE

Determine the effects of a novel nutritional supplement (NNS) on aging defense mechanisms (ADMs) of the skin of healthy adults in response to increasing doses of damaging UVR.

SUBJECTS & METHODS

Inclusion

- Forty healthy non-smoking subjects (n=56 females and n=4 males)
- 40 to 75 years of age
- Fitzpatrick skin types I and II
- Body mass index (BMI) between 15 and 30 (kg/m²)

Exclusion

- Participants with a history of chronic diseases, skin diseases or abnormalities currently under treatment
- Consuming dietary supplements containing carotenoids, vitamin D, EPA, DHA, & resveratrol
- Ate more than 1 fatty fish per week
- Using or having used an anti-aging or treatment skin care product within 30 days of study
- Pregnant, planning to become pregnant, or nursing

The study was approved by an Institutional Review Board and conducted according to Helsinki Declaration. The study was registered on ClinicalTrials.gov (NCT02525224).

RESULTS

Table 1. Number of apoptotic cells from 3 MED dose site and skin carotenoid levels (RIUs) at baseline and after 8 weeks post-supplementation. Data presented as least square means ± standard error of the means in parentheses. *P<0.05 as compared to baseline levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Cells (cell/field)</th>
<th>Females (cell/field)</th>
<th>Males (cell/field)</th>
<th>Skin Carotenoid Levels (RIUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>11.6 ± 1.1</td>
<td>11.3 ± 1.0</td>
<td>14.7 ± 1.1</td>
<td>28,600 ± 2,927</td>
</tr>
<tr>
<td>8 weeks</td>
<td>5.7 ± 0.6*</td>
<td>5.2 ± 0.6*</td>
<td>10.2 ± 0.6</td>
<td>38,775 ± 2,927*</td>
</tr>
</tbody>
</table>

*P<0.05 as compared to baseline levels

Figure 1. Skin photographs and erythema scores of two representative female subjects depicting erythema and inflammation induced by 3 minimal erythema doses (normal skin, 1 MED, 2 MED, 3 MED) at baseline and after 8 weeks supplementation. Note the reduction in the number of apoptotic cell from biopsies of the 3 MED site at 8 weeks.

SUMMARY & CONCLUSIONS

8 weeks supplementation with the NNS led to dramatic protective effects against UVR induced erythema as evidenced by:

- Bolstered ADMs (antioxidant and DNA protection, cellular stress response, and inflammatory balance mechanisms)
- Significant decreases in skin erythema (P<0.05) at all three MED doses
- Significant reduction in the mean number of apoptotic cells, 1.6 at baseline vs. 5.7 cells/mm² (P<0.05) at the 3 MED site, suggesting that the NNS protected against both UVR-induced DNA damage and apoptosis.
- Increased skin carotenoid levels indicative of an increase in antioxidant protection.
- Additional clinical studies are warranted to examine influence of NNS on other ADMs.

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