Total Cellular Protein in Response to UV

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Tradename: AC ExoEternol

Code: 60200

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 90082-61-4 (or) 68132-21-8 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13042

Lot #: N250701O

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Sirius Red/Fast Green Collagen Assay with UV-B Irradiation

Introduction

Collagen is the main protein of connective tissues, such as skin, bone, tendon and ligament, and the most abundant protein in mammals. Collagen accounts for nearly 25% to 35% of the total human protein content. Collagen is a long, fibrous protein that forms bundles called fibers giving structure and support to cells and tissues. Collagen has great tensile strength and is responsible for skin's elasticity and, therefore, its degradation leads to wrinkles that accompany aging.

Similar to collagen, the non-collagenous proteins within the extracellular matrix substantially contribute to healthy skin. Elastin forms an unstructured yet cross-linked network enabling skin to stretch without breaking. Adhesion proteins, such as laminin and fibronectin provide attachments between cells and the extracellular matrix facilitating skin integrity. The synergistic effects of collagen and non-collagen proteins help prevent visible signs of aging.

A Sirius Red/Fast Green Collagen Assay was conducted to assess the *in vitro* protective effect of **AC ExoEternol** against reductions in collagen synthesis and non-collagenous protein levels caused by UV-B irradiation. Excessive exposure to UV-B light (280-315nm) stimulates inflammation, reactive oxygen species, DNA mutations, and disruptions in dermal-epidermal junction integrity, which can exacerbate skin wrinkling and aging.

Assay Principle

Sirius Red is a unique dye that binds specifically to the helical structure of types I through V collagen, while Fast Green binds to non-collagenous proteins. These two dyes work in conjunction to provide a semi-quantitative method to determine the amounts of collagen and non-collagenous proteins in a sample. After staining, the dyes are easily extracted and have optical density (OD) absorptions at 540nm (Sirius Red) and 605nm (Fast Green). These OD values are utilized to calculate collagen and non-collagenous protein levels.

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Materials

- A. **Kit:** Sirius Red/Fast Green Collagen Kit (Chondrex; 9046) *
- B. **Incubation Conditions:** 37°C at 5% CO_2 and 95% Relative Humidity (RH)
- C. **Equipment:** Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Synergy HT Microplate Reader; Accuris UV Transilluminator; Pipettes
- D. **Cell Line:** Normal Human Dermal Fibroblasts (ATCC; PCS-201-012) *
- E. **Media/Buffers:** Fibroblast Basal Medium (ATCC; PCS-201-030) *; Fibroblast Growth Kit (ATCC; PCS-201-041)*: Phosphate Buffered Saline (PBS)
- F. **Culture Plate:** Falcon Flat Bottom 24-Well Tissue Culture Treated Plates
- G. **Software:** Excel Analysis ToolPak (Microsoft)
- H. **Other:** Sterile Disposable Pipette Tips

**Or suitable alternatives, subject to change without notice based off vendor availability.*

Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete media (CM). 0.01% and 0.05% concentrations of **AC ExoEternol** were added to CM and incubated at 37°C with fibroblasts. Following an 18-hour incubation, the media in all wells was replaced with CM and the tissue culture plates were returned to 37°C for 30 minutes. Next, the fibroblasts were irradiated with UV-B light (302nm) at a dose of 75 mJ/cm^2. After UV-B irradiation, the plates were incubated at 37°C for 24 hours, after which the Sirius Red/Fast Green assay was performed.

Media was removed from wells containing adherent fibroblasts and the cells were washed with PBS. 500 µL of a cooled 95% ethanol/5% glacial acetic acid solution was added to the wells and incubated for 10 minutes at room temperature. 200 µL of the Sirius Red/Fast Green dye solution was added to the fixed cell layer and incubated at room temperature for 30 minutes. The dye solution was removed from the cell layer and washed with water. 1 mL of extraction solution was added for color extraction. The optical density was read at 540nm and 605nm on a Synergy HT Microplate Reader.

Three separate experiments were performed with conditions in duplicate and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. The collagen and non-collagenous protein concentrations of fibroblasts was determined by calculations based on the optical density measurements and expressed in µg. Collagen and non-collagenous protein concentrations were calculated with the following formulas:

$$Collagen\ (\mu g) = \frac{[OD\ 540 - (OD\ 605\ \times 0.291)]}{0.0378}$$

$$Non - Collagenous\ Protein\ (\mu g) = \frac{OD\ 605}{0.00204}$$

Results

The data obtained from this study met criteria for a valid assay and the negative control performed as anticipated. UV-B irradiation (75 mJ/cm^2) of untreated fibroblasts elicited reductions in collagen and non-collagenous protein levels. However, fibroblasts treated with **AC ExoEternol** at 0.01% and 0.05% concentrations were able to attenuate the UV-B irradiation reductions in collagen and non-collagenous protein compared to the negative control.

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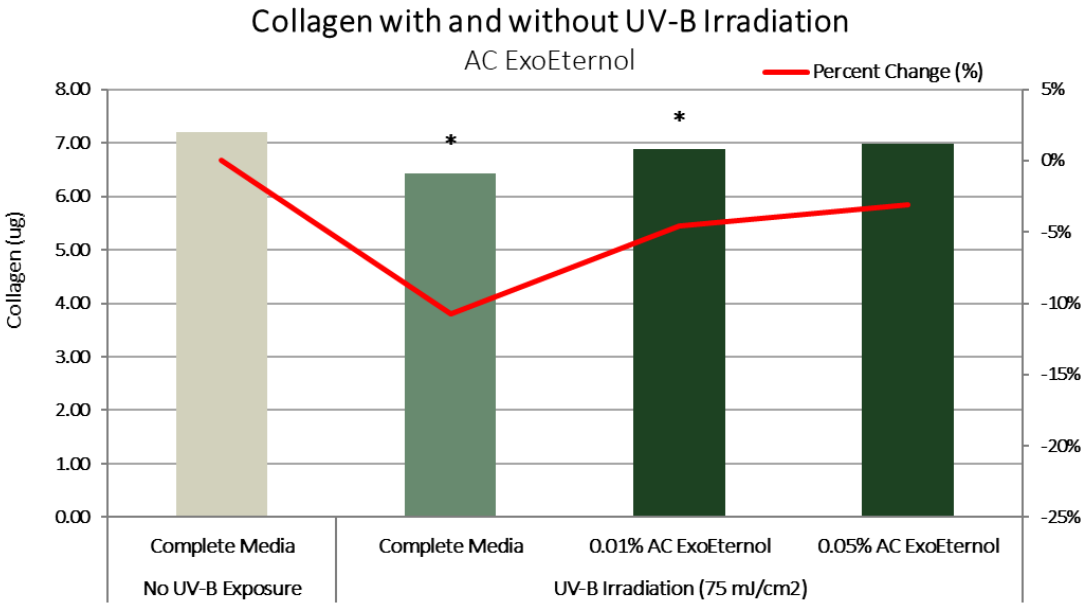

Figure 1. The effect of UV-B irradiation on collagen. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts.

Table 1. Results from one-way ANOVA Statistical Analysis of Collagen Compared to Untreated Fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts.

	0.01% AC ExoEternol	0.05% AC ExoEternol
P-value	0.045*	> 0.05

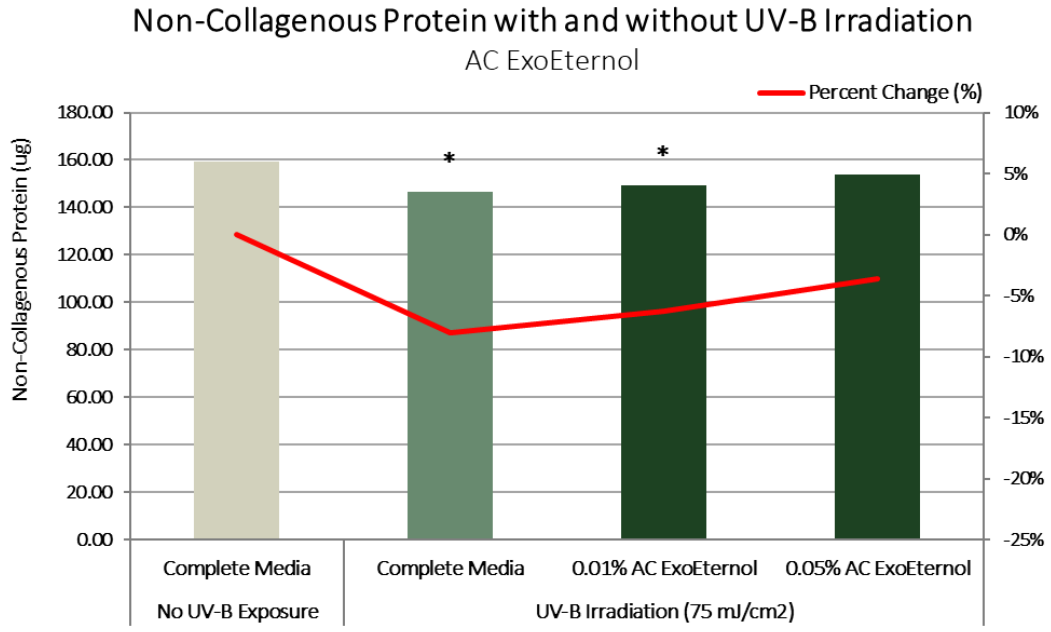

Figure 2. The effect of UV-B irradiation on non-collagenous protein. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts.

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Table 2. Results from one-way ANOVA Statistical Analysis of Non-Collagenous Protein Compared to Untreated Fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts.

	0.01% AC ExoEternol	0.05% AC ExoEternol
P-value	0.011*	> 0.05

Discussion

As shown in Figure 1, UV-B exposure (75 mJ/cm^2) produced an 11% reduction in total collagen synthesis in untreated fibroblasts, demonstrating the negative effects of UV-B light on collagen production. However, AC ExoEternol at 0.01% and 0.05% blunted the negative effects of UV-B irradiation with only 5% and 3% reductions in collagen compared to untreated fibroblasts, respectively. Furthermore, AC ExoEternol at 0.01% and 0.05% had 7% and 9% more collagen production compared to untreated, UV-B irradiated fibroblasts, respectively.

Similarly, as shown in Figure 2, UV-B irradiation produced an 8% reduction in non-collagenous protein of untreated fibroblasts, showing the negative effects of UV-B light on protein levels. However, AC ExoEternol at 0.01% and 0.05% blunted the negative effects of UV-B irradiation with only 6% and 4% reductions in non-collagenous protein compared to untreated fibroblasts, respectively. Furthermore, AC ExoEternol at 0.01% and 0.05% had 2% and 5% more non-collagenous protein compared to untreated, UV-B irradiated fibroblasts, respectively.

Collectively, these data demonstrate that AC ExoEternol attenuates UV-B induced disruptions in collagen synthesis and non-collagenous protein levels, which may lead to improvement in the dermal-epidermal junction integrity, as well as an improved scaffolding matrix, helping prevent visible signs of aging.