Demonstrated Improvement of Prematurely Aged Skin by Oral Intake of TA-65®

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Abstract:

Telomere attrition is the central hallmark of cellular senescence. TA-65® is a small molecule that has been shown to prevent telomere attrition. The main objective of this study was to evaluate cosmetic benefits of oral intake of TA-65® in subjects with prematurely aged skin (photo-aged skin). A randomized, double-blind, placebo-controlled study was carried out on thirty-five photo-aged subjects for sixteen weeks. Clinical assessments were performed at baseline and following four, eight and sixteen weeks of use. The VISIA® complexion analysis system (Canfield Scientific, Fairfield, NJ) showed that TA-65® may reduce pre-clinical damage, uneven pigmentation and wrinkles after eight weeks' daily capsule use. Histological analyses of biopsies revealed that the expression of inflammatory cytokines declined, whereas elastin level increased after sixteen weeks' daily intake of TA-65® capsules, demonstrating the improvement of pre-maturely aged skin.

Introduction:

Repeated exposure of ultra violet light results in photo-aging. In aging and photo-aging, human skin accumulates senescent keratinocytes and fibroblasts. Senescence not only limits the replicative potential of cells but also fuels inflammation associated with aging and photo-aging (Lasry and Ben-Neriah 2015). Thus anti-senescence compounds have tremendous potential as novel therapeutics. TA-65® is a small molecule telomerase activator derived from the *Astragalus* plant and has the ability to upregulate basal telomerase activity as well as reduce telomere attrition (Harley, Liu et al. 2011). This study was designed to test cosmetic skin improvement following the oral intake of TA-65®.

Objective:

The main objective of this study was to evaluate the cosmetic benefits of TA-65® capsules for skin care in subjects with prematurely aged skin.

Methodology:

A randomized, double-blind, placebo-controlled study of thirty-five photo-aged subjects was carried out for sixteen weeks with clinical assessment performed at baseline and following four, eight and sixteen weeks of use.

The subjects were randomly assigned to either the placebo group or the treatment group. The placebo group took two placebo capsules per day for sixteen weeks. The treatment group took two TA-65® capsules (250 units each) per day for sixteen weeks. The placebo capsule did not contain the active ingredient, TA-65®, but is otherwise identical in formulation and packaging to the TA-65® capsule.

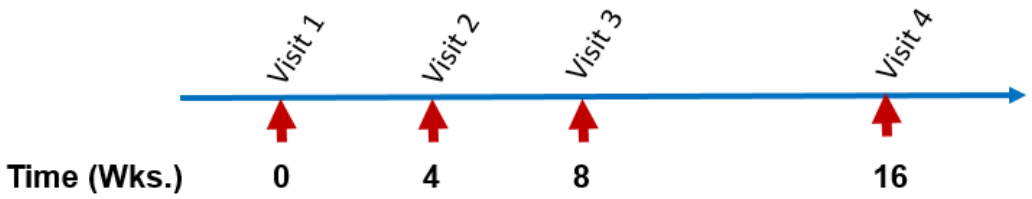

Figure 1: Visits at different time during study period

VISIA® complexion analysis system

The VISIA® complexion analysis system (Canfield Scientific, Fairfield, NJ) was used to objectively measure (Goldsberry, Hanke et al. 2014) wrinkles and brown spots at all indicated visits (Figure 1).

Punch biopsy:

One subject from each group underwent a punch biopsy procedure, wherein a small skin tissue sample was taken at the beginning and end of the trial (16 weeks). The biopsy samples (3mm) were sent to the laboratories of Development Engineering Sciences, LLC (Flagstaff, AZ) which analyzed the molecular markers suggestive of the skin's health by nuclear counting, histology and Real Time – Polymerase Chain Reaction (RT-PCR). The following test methods were performed on the punch biopsy samples:

Nuclear counting:

The nuclear counting algorithm was used to count the number of cells present in the stratum basale and spinosum of the H&E stained sections. The algorithm was tuned per Aperio's algorithm user guide for nuclear quantification. Images acquired from the Hamatsu Nanozoomer digital slide scanner were analyzed using Aperio algorithms.

Immunohistochemistry (IHC) analysis:

Collagen 1, elastin and filaggrin presence in the dermis and epidermis, respectively, were quantified using a color deconvolution algorithm from digitally-scanned IHC slides. Collagen 1 was analyzed at a depth of 200μm below the stratum basale, into the dermis. Elastin was analyzed at a depth of 200μm above the hypodermis into the dermis. Filaggrin was measured in the stratum granulosum layer.

Real-time PCR (RT-PCR):

IL-6, IL-8, TNF-α, MMP-1 and MMP-12 were measured by RT-PCR. Samples were analyzed in triplicate to calculate fold change (increase or decrease) in gene expression.

Results and Discussion:

The VISIA® complexion analysis system analyzed wrinkles and brown spots.

Compared to baseline, there was a significant reduction in the mean scores of wrinkles after taking TA-65® capsules for eight weeks (33% decline; p=0.02). The mean scores (S.E) at baseline and after eight weeks were 21±2 and 14±2 respectively (Figure 2A). This result indicates that oral intake of TA-65® capsule can effectively reduce facial wrinkles.

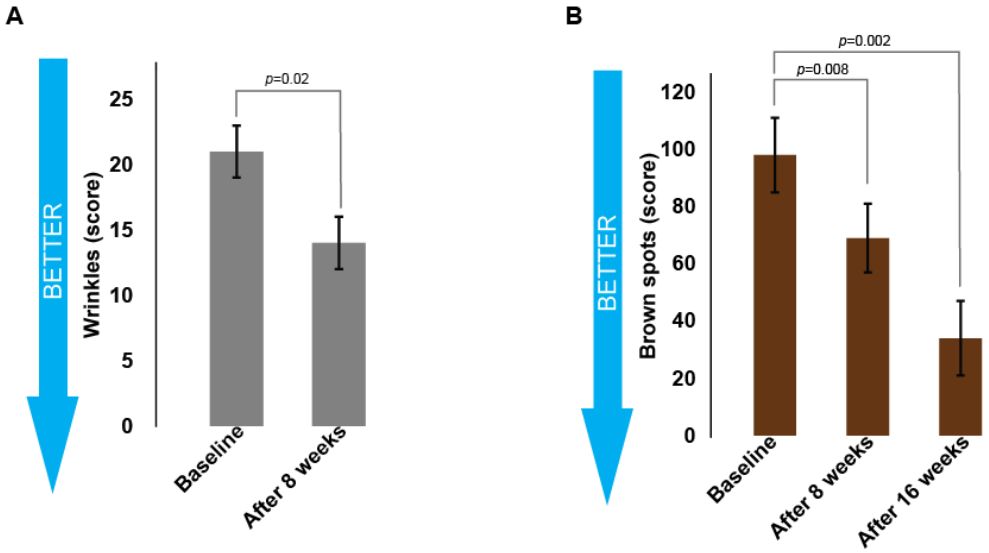

Figure 2: Cosmetic benefits of TA-65® capsule. Daily intake of TA-65® capsule reduced wrinkles by 33% after 8 weeks (A). Brown spots declined by 30% after 8 weeks and 65% after 16 weeks compared to baseline (B). *p*-values are estimated by the t-test.

There was a significant reduction in the mean scores of brown spots after the oral intake of TA-65® capsules for eight weeks (30% decline; p=0.008) and sixteen weeks (65% decline; p=0.002). The mean scores (S.E) at baseline, eight weeks and sixteen weeks were 98±13, 69±12 and 34±13, respectively (Figure 2B). These results indicate that oral intake of TA-65® capsules can diminish uneven pigmentation.

Histopathology Findings

Histopathology comparisons between active vs. placebo treatment sites at the sixteen week time point did not reveal any noticeable differences with respect to acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration, or dermal edema. No trend change existed for prominence of any of these characteristics between active and placebo treated samples. Representative histopathology is shown in figure 3.

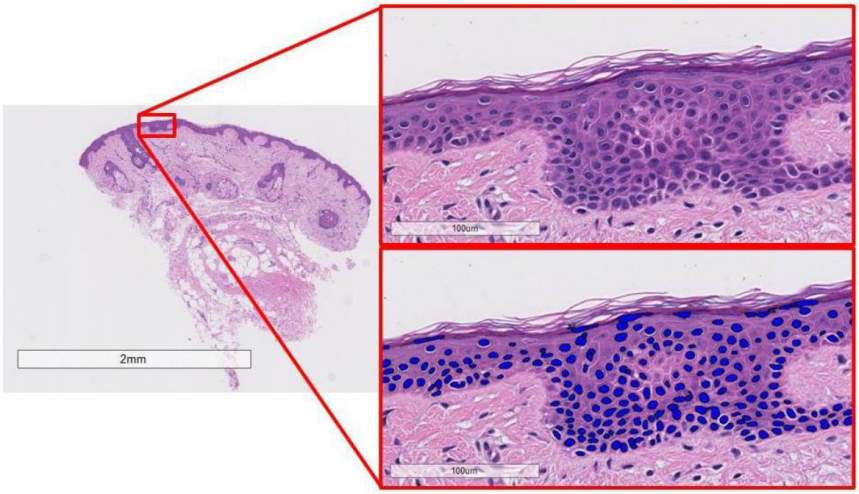

Figure 3: H&E staining of punch biopsy at baseline in TA-65® group. No acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration or dermal edema were observed at baseline. Left panel is the low magnification (scale bar = 2 mm) and right panel is the high magnification (scale bars = 100μm)

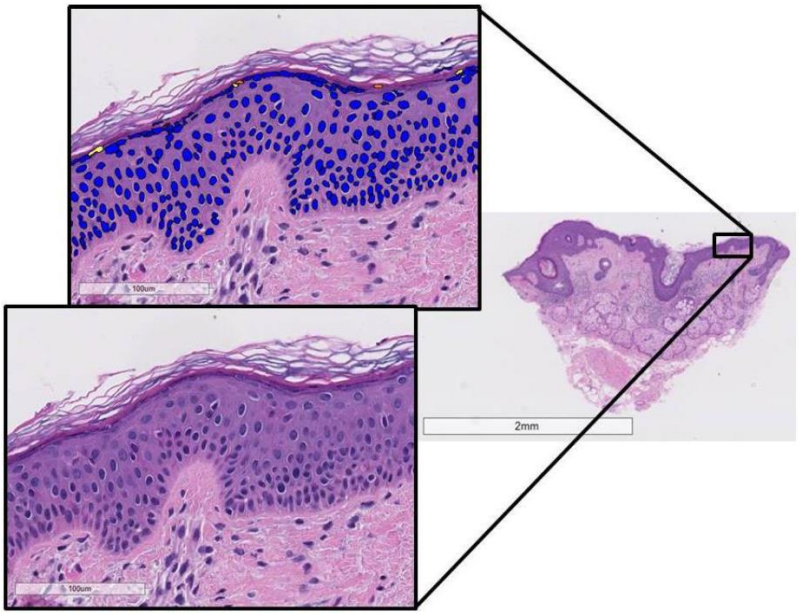

Figure 4: H&E staining of punch biopsy after sixteen weeks' intake of TA-65® capsules. No acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration or dermal edema were observed with 16 weeks' use of *TA-65®* capsules Right panel is the low magnification (scale bar = 2mm) and left panel is the high magnification (scale bars = 100μm).

Immunohistochemistry and RT-PCR

Expression of pro-inflammatory molecules IL-6 and IL-8 increased in the skin of the subjects who took placebo capsules, whereas oral intake of TA-65® capsules reduced them within sixteen weeks (Figure 5A). A twofold reduction in the IL-6 and 6 fold reduction in the IL-8 were observed after sixteen weeks' intake of TA-65® capsules; in the placebo group, there is a tenfold increase in IL-6 and thirtyfold increase in IL-8. No improvement in the molecular markers TNF-α, MMP-1 and MMP-12 were observed following the oral intake of TA-65® capsules.

Elastin is a structural protein found in the dermis that provides flexibility to the skin and allows for it to elastically return to its native resting architecture. Figure 5B shows that the oral intake of TA-65® capsules increases the level of elastin by 93% after sixteen weeks. No improvement in the molecular markers collagen and filaggrin were observed following the oral intake of TA-65® capsules.

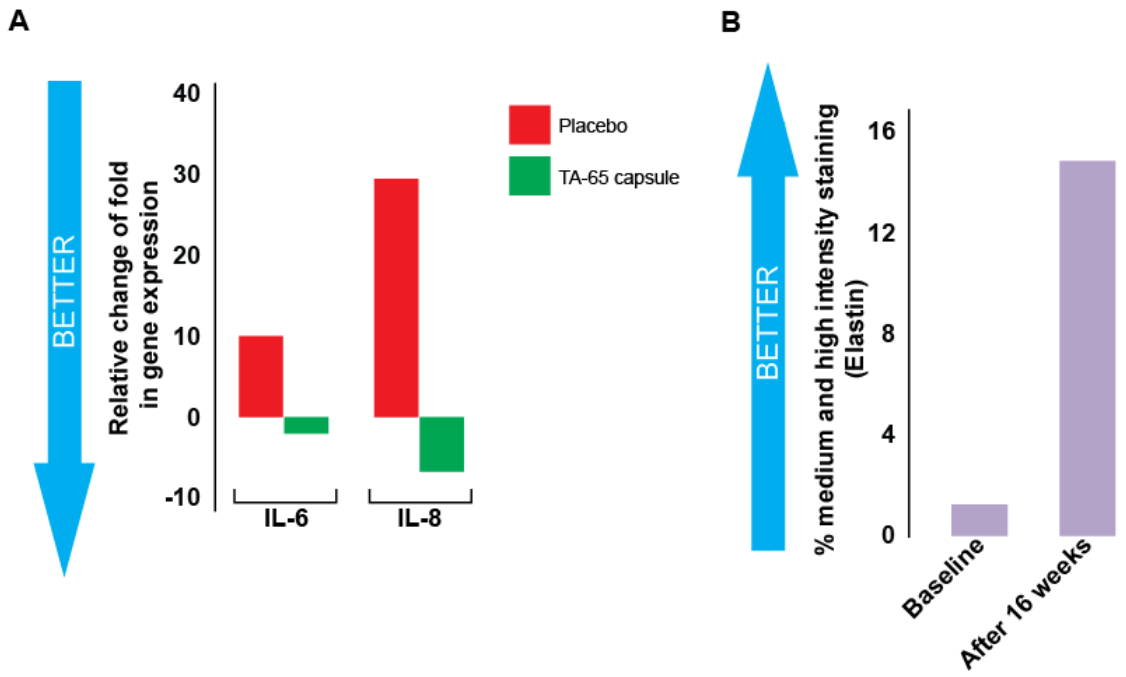

Figure 5: Expression of inflammatory cytokines declines after 16 weeks' use of TA-65®. IL-6 and IL-8 declines by twofold and sevenfold after sixteen weeks' oral intake of TA-65® capsule. (A). Elastin level increases by 93% after sixteen weeks' oral intake of TA-65® capsules (B).

References:

Goldsberry, A., C. W. Hanke and K. E. Hanke (2014). "VISIA system: a possible tool in the cosmetic practice." J Drugs Dermatol **13**(11): 1312-1314.

Harley, C. B., W. Liu, M. Blasco, E. Vera, W. H. Andrews, L. A. Briggs and J. M. Raffaele (2011). "A natural product telomerase activator as part of a health maintenance program." Rejuvenation Res **14**(1): 45-56.

Lasry, A. and Y. Ben-Neriah (2015). "Senescence-associated inflammatory responses: aging and cancer perspectives." Trends Immunol.