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Tradename: AC PomeaShield

Code: 16935

CAS #: 7732-18-5 & 84961-57-9 & 1686112-36-6 (or) 68333-16-4

Test Request Form #: 9266

Lot #: N220316M

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigator: *Daniel Shill*

Test Performed:
Cellular Detoxification / Autophagy Detection Assay

Introduction

Autophagy is the self-identification and delivery of damaged cellular proteins and organelles, intracellular microbes, and toxins to lysosomal vacuoles for breakdown. The byproducts of this process are either excreted from the cell, utilized for energy production, or to support catabolic pathways. This removal of cellular damage regulates skin cell survival and function, as a reduction in autophagy (i.e. an accumulation of cellular damage) increases inflammation and reactive oxygen species, perturbs metabolism, and promotes senescence, all of which are hallmarks of aging at the cellular level.

An Autophagy Detection Assay was conducted to assess the *in vitro* effect of AC PomeaShield to trigger autophagy in dermal fibroblasts. Activating this biological detoxification process maintains skin homeostasis and counteracts the age-related decline in skin cell function.

Assay Principle

The Green Detection Reagent, supplied in Abcam's Autophagy Detection Kit, fluoresces when bound to autophagic-lysosomal vacuoles produced during autophagy to indicate autophagic activity, while the Nuclear Stain fluoresces when bound to nuclear DNA to indicate cellular nuclei. These two dyes work in conjunction to provide a specific and quantitative method for determining autophagic activity. By displaying the Green Detection Reagent fluorescent signal as a function of the Nuclear Stain fluorescent signal, autophagic activity at the cellular level can be quantified and normalized.

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Materials

- **A. Kit:** Autophagy Detection Kit (Abcam; ab139484)*
- **B. Incubation Conditions:** 37°C, 5% CO_2, and 95% relative humidity (RH)
- **C. Equipment:** Forma humidified incubator, ESCO biosafety laminar flow hood, Synergy HT Microplate reader; Pipettes; Light microscope
- **D. Cell Line:** Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)*
- **E. Media/Buffers:** Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Dimethyl Sulfoxide (DMSO); 10x Assay Buffer (ab139484)*; Deionized water
- **F. Reagents:** Rapamycin (ab139484)*; Chloroquine (ab139484)*; Nuclear Stain (ab139484)*; Green Detection Reagent (ab139484)*
- **G. Culture Plate:** 96 Well Black Side/Clear Bottom Tissue Culture Treated Microplates
- **H. Other:** Sterile disposable pipette tips

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

Method

Human dermal fibroblasts were seeded into a 96-well tissue culture microplate and grew to 50%-70% confluency in complete media (CM). 0.01%, 0.1% and 1.0% concentrations of **AC PomeaShield** were added to complete media, while rapamycin and chloroquine were initially reconstituted in DMSO and deionized water, respectively, and further diluted with CM. As experimental controls, chloroquine at 40 µM was utilized as a negative control to inhibit autophagic-lysosomal vacuole development, and rapamycin at 10 µM was utilized as a positive control to augment autophagic-lysosomal vacuole development. Testing conditions were added to fibroblasts and incubated at 37°C. Following an 18-hour incubation, the media in all wells was removed and cells were washed twice with 1x Assay Buffer. The Nuclear Stain and Green Detection Reagent were diluted in CM and added to all wells. After a 30-minute incubation at 37°C, the Nuclear Stain and Green Detection Reagent were removed from all wells, and cells were washed with 1x Assay Buffer. Next, fluorescence measurements were taken with the following wavelengths (excitation / emission): Nuclear Stain (350 nm / 461 nm) and Green Detection Reagent (463 nm / 534 nm).

To account for differences in cell counts, Cellular Detoxification values are expressed as the Autophagic-Lysosomal Vacuoles Signal (Green Detection Reagent) divided by the Nuclear Signal (Nuclear Stain), as calculated by the following equation:

$$Cellular\ Detoxification = \frac{Autophagic/Lysosomal\ Vacuoles\ Signal}{\text{Nuclear Signal}}$$

Percent change is expressed relative to CM and calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{RFU_{CM} - RFU_{Sample}}{RFU_{CM}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated. Compared to untreated fibroblasts, chloroquine (40 µM) reduced autophagic-lysosomal vacuoles whereas rapamycin (10 µM) increased autophagic-lysosomal vacuoles. Fibroblasts treated with **AC PomeaShield** at 0.01%, 0.1%, and 1.0% demonstrated increased autophagic activity compared to untreated fibroblasts.

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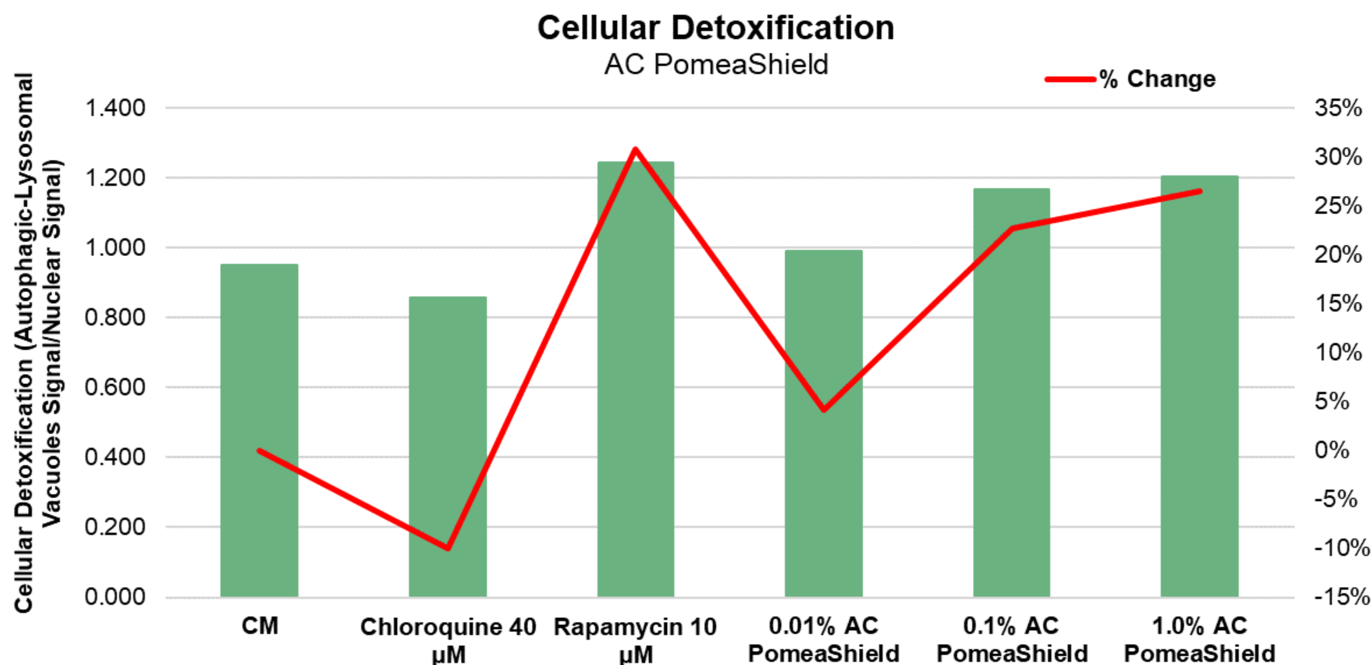

Figure 1. The effect of AC PomeaShield on fibroblast cellular detoxification.

Discussion

As shown in Figure 1, fibroblasts incubated with chloroquine, a known autophagy inhibitor, exhibited a 10% reduction in autophagic-lysosomal vacuoles compared to untreated fibroblasts. Conversely, fibroblasts exposed to rapamycin elicited a 31% increase in autophagic-lysosomal vacuoles compared to untreated fibroblasts. These data demonstrate cellular detoxification in fibroblasts is dynamic and can be manipulated with exogenous compounds.

Similarly, fibroblasts treated with AC PomeaShield at 0.01%, 0.1%, and 1.0% demonstrated 4%, 23%, and 27% increases in autophagic-lysosomal vacuoles compared to untreated fibroblasts, respectively. These data demonstrate AC PomeaShield activates fibroblast detoxification.

Collectively, autophagy is a biological process that identifies toxins within the cell and removes or recycles damaged cellular components to maintain skin homeostasis. These data indicate AC PomeaShield triggers this cellular detoxification process, which may help to attenuate characteristics of cellular aging.

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