IL-1α ELISA

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Tradename: AC Skinmuni-Tea

Code: 12048

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 84650-60-2 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 8695

Lot #: N210716E

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

Test Performed:
Interleukin (IL)-1α Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

IL-1α is a pro-inflammatory cytokine that is largely responsible for the initial inflammatory response and induces the production of other inflammatory mediators including tumor necrosis factor-α (TNF-α). In keratinocytes, chronically elevated IL-1α levels can lead to hair loss, scaling, and a reduction in skin elasticity. Furthermore, epidermal inflammatory cytokines have been shown to elicit a systemic inflammatory response and accelerate cellular aging if left unattended.

An IL-1α ELISA was conducted to assess the *in vitro* effect of **AC Skinmuni-Tea** on IL-1α concentrations produced by keratinocytes in a pro-inflammatory environment.

Assay Principle

The Abcam IL-1α ELISA Kit operates by mixing an affinity tag labeled capture antibody with a reporter conjugated detector antibody that binds to IL-1α. When the capture and detector antibodies immunocapture IL-1α, a complex is formed that becomes immobilized when bound to the anti-tag antibodies coating the wells. Unbound materials are removed during washing steps, and addition of the 3,3',5,5'-tetramethylbenzidine (TMB) Development Solution generates a blue color that is catalyzed by horseradish peroxidase (HRP). Adding the Stop Solution to the samples finalizes the color change from blue to yellow and the absorbance is measured. The signal generated is proportional to the amount of bound IL-1α and concentrations are calculated.

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Materials

A. Kit:	Human IL-1α ELISA Kit (Abcam, ab178008)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity
C. Equipment:	Forma Humidified Incubator; ESCO Biosafety Laminar Flow Hood; Synergy HT Microplate Reader; Pipettes; Light Microscope
D. Cell Line:	Normal Human Epidermal Keratinocytes (ATCC; PCS-200-011)*
E. Media/Buffers:	Keratinocyte Basal Medium (ATCC; PCS-200-030)*; Keratinocyte Growth Kit (ATCC; PCS-200-040)*; Deionized water
F. Reagents:	Lipopolysaccharide (LPS) (1.0 µg/mL); Dexamethasone (DEX) (10 µM)
G. Culture Plate:	12-Well Flat Bottom Tissue Culture Treated Plates
H. Other:	Sterile disposable pipette tips

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

Methods

Human epidermal keratinocytes were seeded into a 12-well tissue culture plate and grown to confluency in complete media. 0.01%, 0.1% and 1.0% concentrations of **AC Skinmuni-Tea** were added to Complete Media containing 1.0 µg/mL LPS (LPS) and incubated with keratinocytes for 48 hours. LPS was employed to create an inflammatory environment and 10 µM DEX in the presence of LPS (LPS + DEX) was implemented as a positive control to suppress inflammation. After 48 hours, media from all wells was removed and utilized in the Human IL-1α ELISA Kit (ab178008).

Standards were prepared in concentrations ranging from 0.0 pg/mL to 250 pg/mL. After adding 50 µL of standards and samples to the appropriate wells, 50 µL of the Antibody Cocktail was added to all wells. Following a one-hour incubation at room temperature, all wells were washed three times with 350 µL of 1x Wash Buffer PT and 100 µL of TMB Development Solution was added to each well. After a 10-minute incubation in the dark, 100 µL of Stop Solution was added to each well and the optical density was read at 450 nm.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-1α concentration of **AC Skinmuni-Tea** treated-keratinocytes was determined by extrapolation from the standard curve and expressed in pg/mL. Percent changes in IL-1α are relative to LPS and were calculated with the following formula:

$$Percent\ Change\ (\%) = \frac{IL\text{-}1\alpha\ Concentration_{Sample} - IL\text{-}1\alpha\ Concentration_{LPS}}{IL\text{-}1\alpha\ Concentration_{LPS}} \times 100$$

Three separate cellular experiments were performed with conditions in duplicate. Duplicates of IL-1α concentrations from each replicate were averaged. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained from this study met criteria for a valid assay and the experimental controls performed as anticipated. Compared to untreated keratinocytes, LPS increased IL-1α concentrations. LPS + DEX reduced LPS-induced IL-1α levels. Similarly, keratinocytes treated with **AC Skinmuni-Tea** at 0.01%, 0.1%, and 1.0% reduced IL-1α concentrations.

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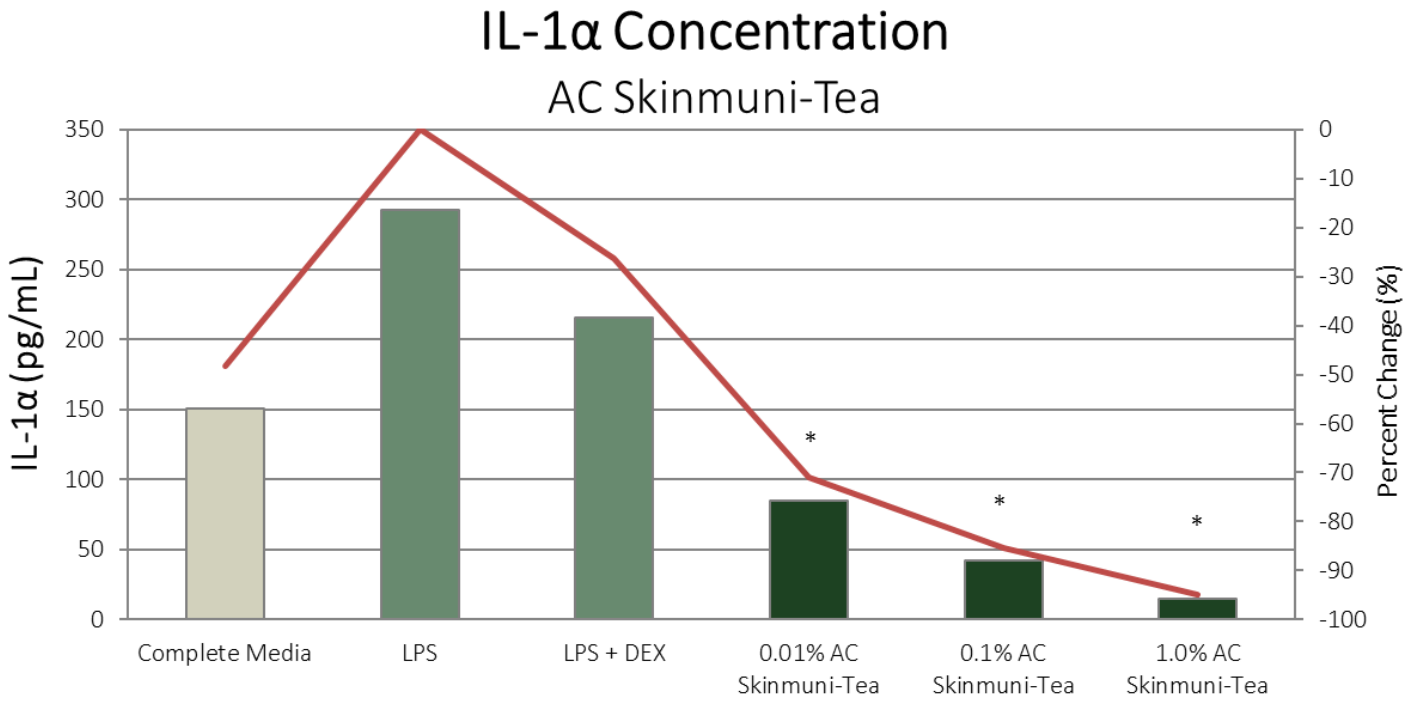

Figure 1. The Effect of **AC Skinmuni-Tea**-treated Keratinocytes on IL-1α Concentrations. * indicates significance ($p < 0.05$) compared to LPS-treated keratinocytes.

Table 1. Results from one-way ANOVA Statistical Analysis. * indicates significance ($p < 0.05$) compared to LPS-treated keratinocytes.

	0.01% **AC Skinmuni-Tea**	0.1% **AC Skinmuni-Tea**	1.0% **AC Skinmuni-Tea**
P-value	< 0.001*	< 0.001*	< 0.001*

Discussion

As shown in Figure 1, keratinocytes exposed to LPS, a known inflammatory stimulus, exhibited a 94% increase in IL-1α concentrations compared to untreated keratinocytes. Conversely, keratinocytes co-incubated with LPS and DEX, a known anti-inflammatory agent, elicited a 26% reduction in IL-1α compared to LPS alone. These data demonstrate IL-1α production in keratinocytes is dynamic and can be manipulated with inflammatory and anti-inflammatory compounds.

Similarly, keratinocytes treated with **AC Skinmuni-Tea** at 0.01%, 0.1%, and 1.0% demonstrated 71%, 85%, and 95% reductions in IL-1α levels compared to LPS-treated keratinocytes, respectively. These data demonstrate **AC Skinmuni-Tea** has anti-inflammatory properties.

Epidermal inflammation can lead to the release of pro-inflammatory cytokines into the circulation and impact cells systemically through paracrine or endocrine signaling. These data indicate **AC Skinmuni-Tea** retains soothing and anti-inflammatory properties, which may help to attenuate the formation of an inflammatory environment and blunt the characteristics of cellular aging.