Intracellular Calcium Assay

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Tradename: AC AmaraSense

Code: 12011

CAS #: 7732-18-5 & 84775-66-6 & 84082-82-6 & 84012-14-6 & & 1686112-36-6 (or) 68333-16-4 (or) 9015-54-7

Test Request Form #: 8730

Lot #: N220829D

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

Test Performed:
Intracellular Calcium Assay

Introduction

Intracellular calcium concentrations, often linked to light exposure, play a key role in skin health and immune response as it can help reduce inflammation, with a direct effect on tissue repair and keratinocyte function. Calcium also helps maintain skin moisture levels and promotes the production of antioxidants.

Keratinocytes, making up 90-95% of the epidermis, are a physical and chemical barrier for the skin by minimizing UV radiation damage, water loss, and microbial, viral, and parasitic invasion. Accordingly, keratinocytes must progressively proliferate, differentiate, and migrate to maintain skin barrier integrity and homeostasis. Provided keratinocyte proliferation, differentiation, and migration are calcium dependent, intracellular calcium levels can serve as a surrogate for skin health.

A Fluo-4 Direct™ Calcium Assay was performed to assess changes in intracellular calcium levels in **AC AmaraSense**-treated human epidermal keratinocytes *in vitro*.

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Assay Principle

The Fluo-4 Direct™ Calcium Assay Kit detects intracellular calcium by utilizing Fluo-4, a cell-permeant fluorescent dye. Upon entering the cell and binding to free calcium, Fluo-4 exhibits fluorescence signals when excited at 488nm providing an indication of intracellular calcium. A suppression dye, which reduces non-intracellular fluorescence and inhibits extracellular transport, is included to eliminate background fluorescence from the complete media and prevent the movement of Fluo-4 out of the cell. An increase in fluorescent signal intensity generated by labeled calcium molecules is indicative of higher levels of intracellular calcium.

Materials

A. Kit:	Fluo-4 Direct™ Calcium Assay Kit (ThermoFisher; F10471)
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 H1 Microplate Reader; Pipettes
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)
F. Culture Plate:	Falcon Flat Bottom 96-Well Tissue Culture Treated Plates
G. Reagents:	Calcium chloride ($CaCl_2$) (Carolina; 85-1800); Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) (EMD Millipore; 32-462-6)
H. Other:	Sterile disposable pipette tips

Methods

Human epidermal keratinocytes were seeded into a 96-well tissue culture plate and allowed to grow to confluency in complete media (CM). 0.01%, 0.1%, and 1.0% concentrations of **AC AmaraSense** were added to complete media and incubated with keratinocytes. As experimental controls, complete media containing 1mM $CaCl_2$ was utilized as a positive control to increase intracellular calcium levels, and 2mM EGTA in the presence of 1mM $CaCl_2$ was utilized as a negative control to reduce the $CaCl_2$-induced increases in intracellular calcium. After a 60-minute incubation at 37°C, 50µL of 2X Fluo-4 Direct™ calcium reagent loading solution was added to all wells and the plate was returned to 37°C for 60 minutes followed by 30 minutes at room temperature. Next, fluorescence measurements (excitation 494nm, emission 516nm) were taken on a Synergy H1 Microplate Reader. Intracellular Calcium results are shown as Relative Fluorescence Units (RFU) and expressed as percentage change, calculated by the following equation:

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

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Results

The data obtained from this study met criteria for a valid assay and the experimental controls performed as anticipated. Keratinocytes treated with **AC AmaraSense** at 0.01%, 0.1%, and 1.0% augmented intracellular calcium levels compared to complete media alone.

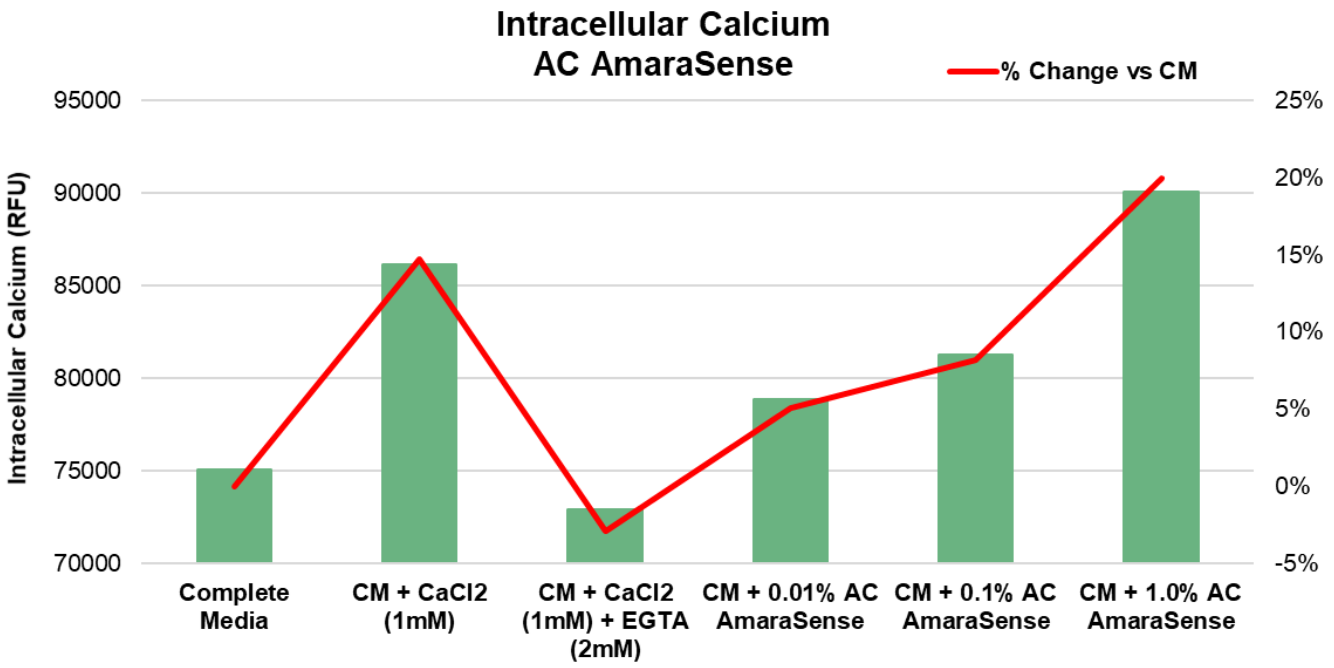

Figure 1: Effect of **AC AmaraSense** on Keratinocyte Intracellular Calcium Levels.

Discussion

As shown in Figure 1, adding $CaCl_2$ to complete media increased keratinocyte intracellular calcium by 15% compared to complete media alone. However, when EGTA was introduced to $CaCl_2$ and complete media, intracellular calcium was reduced by 16% compared to $CaCl_2$ and complete media, establishing our experimental model. **AC AmaraSense** at 0.01%, 0.1%, and 1.0% enhanced keratinocyte intracellular calcium levels by 5%, 8%, and 20% compared to complete media alone, respectively.

Collectively, these data demonstrate that **AC AmaraSense** increases intracellular calcium, an indicator of keratinocyte proliferation, differentiation, and migration, which contributes to the maintenance of skin barrier integrity and homeostasis.