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TGF-β1 ELISA Analysis

Tradename: ACB Pisum Sativum Peptide

Code: 16810

CAS #: 90082-41-0

Test Request Form #: 1460

Lot #: 40996

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Transforming Growth Factor β1 (TGF-β1) Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Transforming Growth Factor beta (TGF-β) is a pleiotropic cytokine which exists in five isoforms, known as TGF-β 1-5, with homologies of 70-80%[1]. TGF-β1 is the most abundant isoform and is highly conserved, with 100% sequence homology between the human, simian, bovine, porcine, and chicken proteins and 99% homology between the human and murine proteins[1]. TGF-β plays a critical role cell cycle regulation and apoptosis. Male pattern baldness is an apoptosis-driven process resulting in early entry into the catagen hair cycle phase[2]. It has also been shown that TGF-β1 expression is highest in the late anagen phase and early catagen phase suggesting an important role in hair cycle regulation[3]. Inhibition of TGF-β is believed to slow regression into the catagen hair cycle phase and result in follicle and hair shaft retention and prevention of hair loss[4].

Transforming Growth Factor-β ELISA was conducted to assess the changes in TGF-β levels in **ACB Pisum Sativum Peptide**-treated *in vitro* cultured Normal Human Dermal Fibroblasts.

1. Human/Mouse TGF beta 1 ELISA Ready-SET-Go! (Second Genetration). *eBioscience*® (2009)
2. Yumika Tsuji, *et. al.*. A Potential Suppressor of TGF-β Delays Catagen Progression in Hair Follicles. *JID Symposium Proceedings*, 8: 65-68 (2003)
3. Kerstin Foitzik, *et. al.* Control of the murine hair follicle regression (catagen) by TGF-β1 *in vivo*. *FASEB J,* 14: 752-760 (2000)
4. Roberta Mazzieri, *et al.* Expression of a truncated latent TGF-β-binding protein modulates TGF-β signaling. *J. Cell Sci.118: 2177-2187 (2005)*

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Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to Human/Mouse TGF-β1. The Human/Mouse TGF-β1 ELISA recognizes the mature/active form of TGF-β1 without association with Latency Associated Peptide (LAP). The samples require acid-treatment and neutralization to remove LAP from TGF-β1 prior to evaluation. Antibodies specific for TGF-β1 epitopes are coated on a microtiter plate. In positive samples, TGF-β will bind to these antibodies and are tagged a second time with another TGF-β-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen/substrate solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of TGF-β1 in each sample.

Materials

A. Kit:	Human/Mouse TGF-β 1 ELISA Kit (eBioscience; 88-8350)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes; Centrifuge
D. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers:	Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
F. Culture Plate:	Falcon flat bottom 24-well tissue culture treated plates
G. Reagents:	500X Cell Stimulation Cocktail (eBioscience; 00-4970-93); Pirfendione (Sigma Aldrich; P2116-10MG); Hydrochloric Acid (HCL); Sodium Hydroxide (NaOH)
H. Other:	Sterile disposable pipette tips; wash bottles

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Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete serum-free media. 1%, 0.1%, 0.01% concentrations of **ACB Pisum Sativum Peptide** were added to complete serum-free media containing 1X Cell Stimulation Cocktail and incubated with fibroblasts for 72 hours. Complete media containing 1X Cell Stimulation Cocktail was used to create an environment with increased TGF-β secretion and 0.5mg/mL Pirfendione in the presence of the Cell Stimulation Cocktail was used as a positive control to inhibit TGF-β production.

A microtiter plate was coated with capture antibody in coating buffer overnight. Following coating incubation the wells were washed and blocked using assay diluent containing 2% fetal bovine serum provided in the eBioscience kit. As described in the Assay Principle section, the cell culture supernatant samples were acid treated to remove LAP using 1N Hydrochloric Acid and neutralized with 1N Sodium Hydroxide. Standards were prepared in concentrations ranging from 1000pg/mL to 0pg/mL assay diluent, provided in the R&D Systems kit. 100µL of standards, controls, and samples were added to appropriate wells. After an overnight incubation at 4°C, for maximal sensitivity, and washing, 100µL of detection antibody was added to all wells. Following a one hour incubation and washing, 100 µL of Avidin-HRP was added to each well for 1 hour at room temperature. All wells were thoroughly washed and 100µL of substrate solution was added for the colorimetric reaction. 50µL stop solution was added to stop the reaction after 15 minutes. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The TGF-β concentration of **ACB Pisum Sativum Peptide** treated-NHDFs was determined by extrapolation from the standard curve and expressed in pg/mL.

Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

ACB Pisum Sativum Peptide at a concentration of 1% was able to decrease TGF-β1 production.

TGF-β production percent decrease is calculated by the following formula:

$$Percent\ (\%)\ Change = \frac{TGF\beta\ Concentration_{Product} - IGF1\ Concentration_{Stimulated\ Cells}}{IGF1\ Concentration_{Stimulated\ Cells}} \times 100$$

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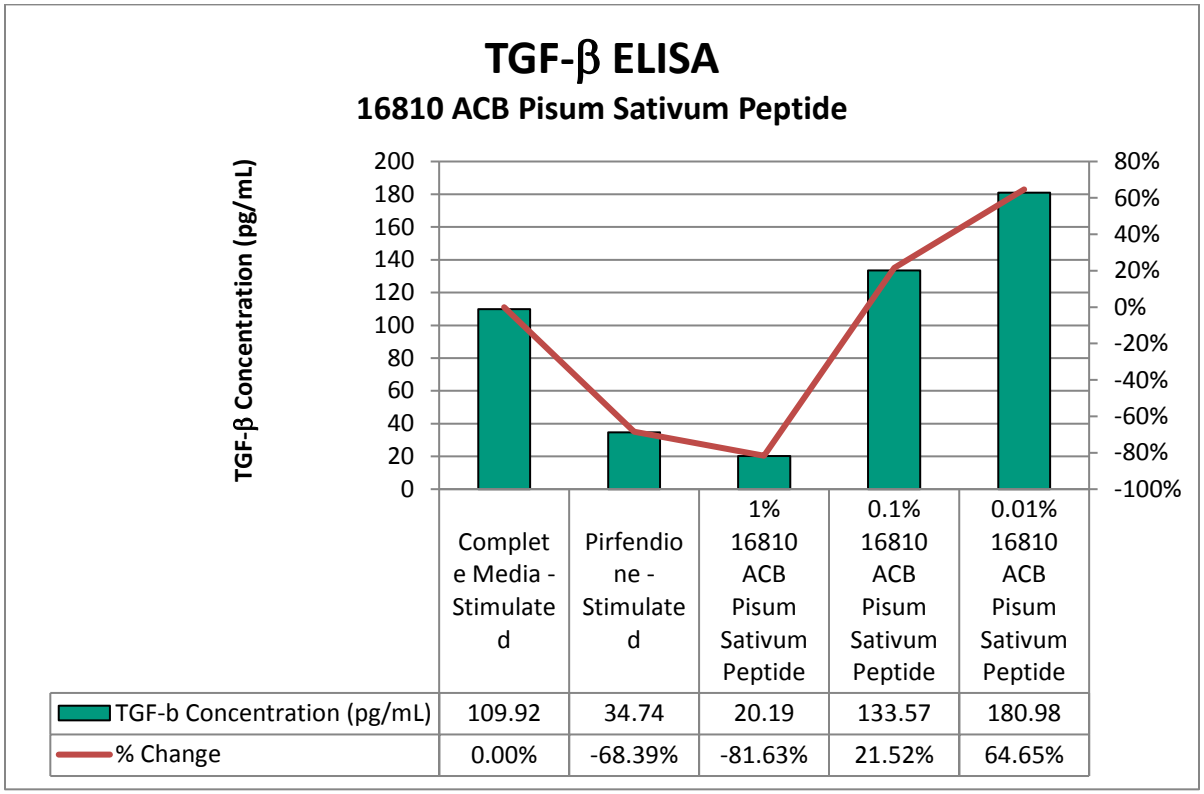

Figure 1: Stimulated and treated NHDF concentrations and percent change

Discussion

As shown in figure 1, **ACB Pisum Sativum Peptide** (code 16810) created an environment conducive to hair growth and follicle stimulation. The decreased concentration of TGF-β should allow for hair shaft retention and maintenance of the follicle in the anagen growth phase. It can therefore be concluded that at normal use concentrations **ACB Pisum Sativum Peptide** can aid in prevention of male pattern baldness.