TGF-β1 ELISA Analysis

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Tradename: AC Hair and Scalp Complex PF

Code: 11301PF

CAS #: 7732-18-5 & 91722-22-4 & 84929-31-7 & 107-43-7 & 107-41-5 & 85251-63-4 & 84650-60-2 & 84775-94-0 (or) 1686112-10-6 & 100684-36-4

Test Request Form #: 5654

Lot #: 67284P

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

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 **AC Hair and Scalp Complex PF** -treated *in vitro* cultured Normal Human Dermal Fibroblasts.

1. Yumika Tsuji, *et. al.*. A Potential Suppressor of TGF-β Delays Catagen Progression in Hair Follicles. *JID Symposium Proceedings*, 8: 65-68 (2003)
2. Kerstin Foitzik, *et. al.* Control of the murine hair follicle regression (catagen) by TGF-β1 *in vivo*. *FASEB J,* 14: 752-760 (2000)
3. 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 TGF-β1. The Human 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-β1 will bind to these antibodies and are tagged a second time with another TGF-β1 -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:**	TGF beta 1 Human ELISA Kit (ab 100647)
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 Primary Fibroblasts (HDFa) (ATCC PCS-201-012)
E.	**Media/Buffers:**	Fibroblast Basal Medium (ATCC PCS-201-030); Fibroblasts Growth Kit- Low Serum (ATCC PCS-201-041) Penicillin-Streptomycin (50U-50mg/mL) Amphotericin (45pg/mL); 20X Wash Buffer Concentrate; 5X Assay Diluent; Biotinylated anti-Human TGF beta 1; Recombinant Human TGF beta 1 Standard; 500X HRP-Streptavidin Concentrate; TMB One-Step Substrate Reagent; Stop Solution
F.	**Culture Plate:**	Falcon flat bottom 12-well tissue culture treated plates
G.	**Reagents:**	500X Cell Stimulation Cocktail (eBioscience; 00-4970-93); Pirfendione (Sigma Aldrich; P2116-10MG); 1N Hydrochloric Acid (HCL); 1.2 N Sodium Hydroxide (NaOH)/0.5 M HEPES
H.	**Other:**	Sterile disposable pipette tips; wash bottles

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Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete serum-free media. 1%, 0.1%, 0.01% concentrations of **AC Hair and Scalp Complex PF** 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-β1 secretion and 0.5mg/mL Pirfenidone in the presence of the Cell Stimulation Cocktail was used as a positive control to inhibit TGF-β1 production.

For cell culture supernate sample preparation, add 0.1 mL 1 N HCL into 0.5 mL cell culture supernate. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.1 mL 1.2 N NaOH/0.5 M HEPES (pH 7.0 – 7.6). Mix tubes thoroughly. Assay immediately. The activated sample may be diluted with 1X Assay Diluent. The concentration read off the standard curve must be multiplied by the dilution factor.

Add 100 µl of each standard 0.082 ng/ml - 60 ng/ml and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µl) using a multi-channel Pipette. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1X Wash Buffer by aspirating. Invert the plate and blot it against clean paper towels. Add 100 µL of 1X Biotinylated TGF beta 1 Detection Antibody to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step. Add 100 µL of 1X HRP-Streptavidin solution to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The TGF-β1 concentration of **AC Hair and Scalp Complex PF** treated-NHDFs was determined by extrapolation from the standard curve and expressed in pg/mL.

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Results

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

AC Hair and Scalp Complex at all concentrations was able to decrease TGF-β1 production. **AC Hair and Scalp Complex** at 1.0% and 0.01% decreased TGF-β1 to concentrations compared to the unstimulated complete media.

TGF-β1 production percent decrease is calculated by the following formula:

$$Percent\ (\%)\ Change = \frac{TGF\beta 1\ \ Concentration_{Product} - TGF\beta 1\ Concentration_{Stimulated\ Cells}}{TGF\beta 1\ Concentration_{Stimulated\ Cells}} \times 100$$

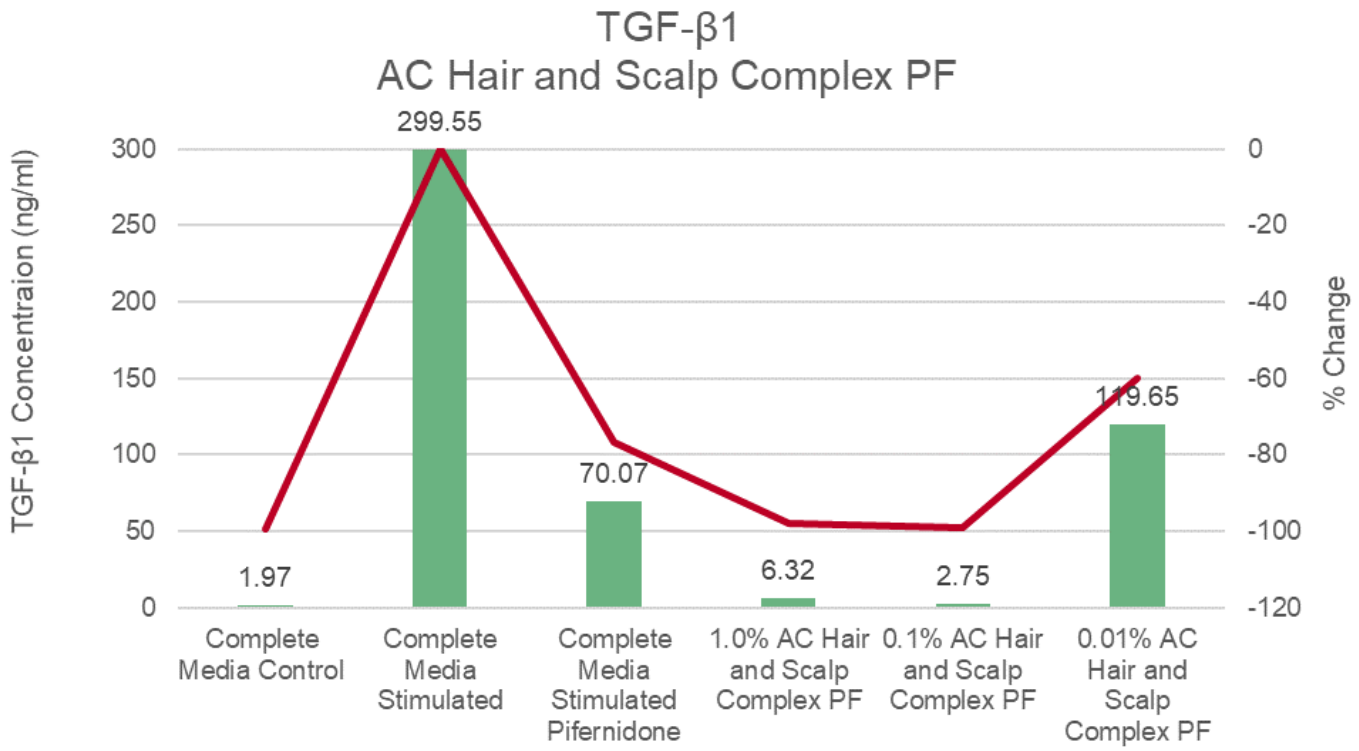

Figure 1: Stimulated and treated NHDF concentrations and percent change

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Discussion

Transforming Growth Factor Beta 1 (TGF-β1) is a family of dimeric proteins that regulate proliferation and apoptosis in many cell types in the body. Previous research shows that TGF-β1 plays a key role in follicle miniaturization, fibrosis, and eventual hair loss. It has been referred to as the "hair follicle assassin" for its deleterious effects on hair follicles. The aim of the study was to suppress/inhibit this protein such that it cannot bind to its receptor and act as a negative growth factor.

As shown in figure 1, **AC Hair and Scalp Complex PF (11301PF)** created an environment conducive to hair growth and follicle stimulation. The decreased concentration of TGF-β1 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 **AC Hair and Scalp Complex PF** can aid in prevention of male pattern baldness.