CBD in Skin Care: Impacts of Time & Tissue Model

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INTRODUCTION

CBD has been hailed as the hottest new ingredient in skin care, with CBD-containing products appearing on the market at a record pace over the past 2 years. CBD products span all areas of the personal care industry, from skin care to hair care to active cosmetics. Claims are diverse and include anti-inflammatory, anti-itch, anti-acne and anti-aging. Despite the widespread use of CBD in topical cosmetic products, there is limited data regarding the effects of cannabinoids in the skin. Most studies have either been carried out using monolayer cell cultures with a single cell type or consumer-based surveys. Studies with 2D cells have shown changes in biological mechanisms that range from regulation of inflammation via NRF2, NFKB and inflammasome pathways, melanogenesis, cell proliferation and apoptosis and keratinocyte differentiation.

In order to establish a testing method for evaluating CBD and CBD-like compounds and formulations, a series of studies was performed to characterize the gene expression response to CBD in the skin. Studies were performed to correlate gene expression changes in an *in vitro* skin model containing keratinocytes and fibroblasts (MatTek EFT-400) with an *ex vivo* skin tissue system (Genoskin NativeSkin).

METHODS

All testing was performed using a purified CBD isolate dissolved in Transcutol CG® (Gattefossé).

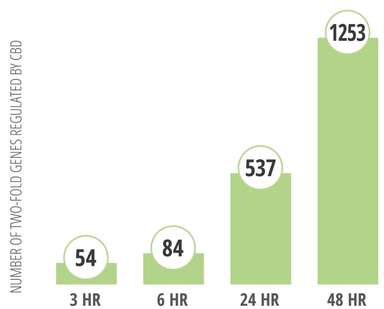
In vitro tissues
In vitro studies were conducted using full-thickness 3D skin equivalents containing epidermal keratinocytes and dermal fibroblasts (MatTek). The CBD solution (5 mg/mL) was applied to the surface of the skin cultures for 3, 6, 24, or 48 hours (N=4). RNA was isolated from the skin cultures. Gene expression was assessed using Affymetrix GeneChip™ microarrays which measure ~20,000 transcripts.

Ex vivo tissues
Ex vivo studies were conducted using adult human abdominal skin explants (Genoskin). The CBD solution (5, 15 or 30 mg/mL) was applied to the surface of the skin cultures for 24, 48, or 72 hours N=3). RNA was isolated using a modified manual RNeasy® Mini (Qiagen) protocol. Gene expression was assessed using Genemarkers qPCR-based CBD Panel which measured 163 genes.

Statistics
T-tests (unpaired, two-tailed) were performed to identify statistically significant changes in gene expression ($p<0.05$).

IN VITRO: CBD-INDUCED GENE EXPRESSION CHANGES OVER TIME

Microarray Analysis Following Application of 5 mg/mL CBD Isolate



Primary Biological Functions Impacted by CBD

- SKIN BARRIER
- IMMUNE/ INFLAMMATION
- LIPIDS
- ANTI-OXIDATION
- EXTRACELLULAR MATRIX

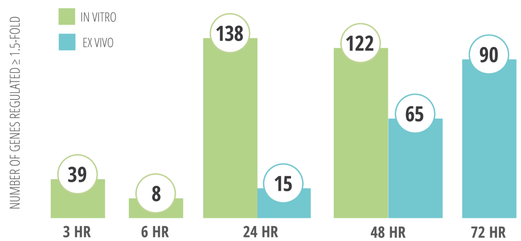
The Expression of 49 Genes Changed Direction Over Time

- 3 ACNE REGULATION
- 1 ANTI-AGING
- 2 ANTIOXIDANT
- 2 CELL RENEWAL / REGENERATION
- 1 CIRCADIAN RHYTHM REGULATION
- 16 EPIDERMAL BARRIER
- 3 EXTRACELLULAR MATRIX BREAKDOWN
- 2 EXTRACELLULAR MATRIX INTEGRITY
- 9 PAIN & INFLAMMATION / IMMUNE RESPONSE
- 3 PIGMENTATION
- 3 SKIN HYDRATION
- 6 TISSUE INTEGRITY / REMODELING
- 1 WOUND HEALING

- Changes in gene expression in response to CBD occur over time, with fewer changes at early time points of 3 and 6 hours
- In the *in vitro* skin tissue model, 24 or 48 hours are the most relevant time points for identifying changes in gene expression
- Gene expression changes are consistent with known functions of CBD
- Expression of some genes changed direction over time, consistent with the fact that gene expression is a dynamic process and analysis of multiple time points may be necessary to obtain a comprehensive activity profile

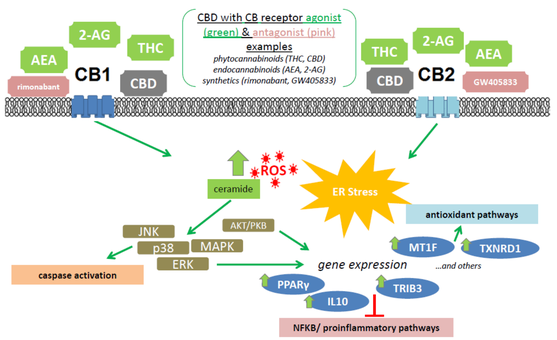
CORRELATION of *IN VITRO* and *EX VIVO* GENE REGULATION

Comparison of the 163 Genes Measured Using the qPCR CBD Panel



Exposure to CBD Regulated the Expression of CB1 and CB2 Receptors in *Ex Vivo* Tissues Only

Activation and Signaling for CB1 & CB2 Receptors



- As expected, higher concentrations and longer exposure times are required to elicit a response in the *ex vivo* skin tissue compared to the *in vitro* skin tissue model
- Similar changes in gene expression occurred in the 2 skin models
- Endogenous expression levels of CB1 and CB2 are low, but detectable changes in expression occurred in the *ex vivo* tissues in response to CBD
- Gene expression analysis is a valuable tool for screening CBD isolates and formulations to identify biological mechanisms of action, verify efficacy and validate claims