SCC76TH ANNUAL SCIENTIFIC MEETING & SHOWCASE
DEC 12 – 14 2022 | JW Marriott LA LIVE, Los Angeles

Bakuchiol and Ethyl (Linoleate/Oleate) Synergistically Modulate Endocannabinoid Tone in Keratinocytes and Repress Type I Interferon, TNF and COX-2 Pathways

Ratan K Chaudhuri[1], William R Swindell[2], Krzysztof Bojanowski[3] & Parvesh Singh[4]

[1]Sytheon, 10 Waterview Blvd, Parsippany, NJ 07054, USA; [2]University of Texas Southwestern Medical Center, Department of Internal Medicine, Dallas, TX, USA; [3]Sunny BioDiscovery Inc., Santa Paula, CA, USA; [4]School of Chemistry, Physics, University of KwaZulu-Natal, P/Bag X54001, Westville, Durban, South Africa.

Background

Interest in cannabidiol (CBD) for topical applications has increased exponentially [1]. The main physiological function of the cutaneous endocannabinoid system (ECS) is to constitutively control the proper and well-balanced proliferation, differentiation, and survival, as well as immune competence and/or tolerance, of skin cells. Research demonstrates that exposing skin to periods of acute physiological stress perturbs the barrier function and delays its recovery. The disruption of this delicate balance might facilitate the development of multiple pathological conditions and diseases of the skin. One of the defenses our bodies possess is a natural ability to fight stress through the ECS. Today, there is compelling evidence that ECS plays an active role in epidermal homeostasis and keratinocyte differentiation [2].

ECS involves four core components: endocannabinoids (eCB), enzymes, transport proteins and receptors. Exocannabinoids include synthetic cannabinoids and plant-derived phytocannabinoids [3]; CBD is one such phytocannabinoid. However, CBD has drawbacks, such as chemical instability at room temperature and conversion under air oxidation to form cannabidiol hydroxyquinone [4]. Isomerization of CBD to tetrahydrocannabinol (THC, forbidden product for topical application) under aqueous acidic conditions has also been reported [5]. The goal of this study was to evaluate the effects of CBD (>99% purity) in keratinocytes (KCs) and reconstituted

human epidermis (RHE) and compare with other phytocompounds, which may regulate ECS activity by targeting ECS regulators such as fatty acid amide hydrolases (FAAH) and fatty acid binding proteins (FABPs) through CB1- and CB2-independent mechanisms.

Materials & Methods

Bakuchiol (BAK, 99.5%) is commercially available from Sytheon (Parsippany, NJ, USA) under the trade name Sytenol® A (INCI: Bakuchiol, CAS # 10309-37-2). Ethyl Linoleate/Oleate (ELN), consisting of approximately 70% linoleate and 15% oleate with other minor fatty acid esters, is also available from Sytheon under the trade name Synovea® EL (INCI: Ethyl Linoleate, CAS # 85049-36-1). Cannabidiol (CBD, 99.7%, CAS # 13956-29-1) was purchased from Nectar Tek, Las Vegas, NV, USA whereas Ethyl Linoleate (EL, >99%, CAS # 544-35-6) from Sigma-Aldrich, USA. A 1:1 blend of BAK and ELN diluted with Isosorbide Dicaprylate (HydraSynol® DOI) is available commercially from Sytheon under the tradename Asyntra® D-Stress.

Molecular docking simulations were performed using Schrödinger software (New York, NY). FABP3, FABP5, FAAH and PTGS2 (COX-2) inhibitory assays were performed using commercial kits. DNA Mmicroarrays were used to evaluate direct effects of each compound on whole genome expression in TNF-stimulated KCs. KCs were incubated with test materials in maintenance medium for 24 hours. Microarray hybridizations were performed using standard protocols by ThermoFisher (Waltham, MA). RT-PCR was performed using RNA from an independent set of biological samples (replication study) with commercial primers and the BioRad iCycler iQ Detection System. DNA motif enrichment upstream of differentially expressed genes was evaluated using a pre-compiled motif dictionary [6] and semiparametric generalized additive logistic models [7]. The effects of test materials on cortisol and IL-8 production were evaluated using RHE tissues (Zen-Bio, Durham, NC). Cortisol was measured using a commercial kit (R&D Systems, Minneapolis, MN) and IL-8 was measured using a sandwich ELISA assay with commercial antibodies (Invitrogen, Waltham, MA; BioLegend, San Diego, CA).

Results & Discussions

Molecular docking simulations showed that BAK and EL each bound the FABP5 active site (ΔG_{bind} -48.4 and -51.02 kcal/mol, respectively), but the 1:1 (w/w) combination BAK+EL bound with highest affinity (ΔG_{bind} -57.6). In contrast, CBD exhibited the weakest binding (ΔG_{bind} -43.8). This BAK-FABP5 interaction has not been demonstrated previously, although

FABP5 was reported to bind retinoic acid [8], which is a functional analog to BAK [9, 10]. Compared to CBD, BAK+ELN was a 3-fold more effective inhibitor of FABP5 and 90-fold more effective in reducing FAAH, with similar inhibitory effects on FABP3 gene expression. This inhibition of FABP5 by BAK or BAK+ELN is expected to amplify eCB tone by slowing AEA or 2-AG degradation.

In TNF-stimulated KCs, BAK+ELN also had potent anti-inflammatory effects, which included inhibition of COX-2 activity, reversal of TNF-induced expression shifts, and reduction of type I interferon pathway and PTGS2 (COX-2) genes. BAK+ELN has anti-inflammatory activity due to synergistic inhibition of eCB degradation (Fig. 1). BAK+ELN may thus offer significant advantages over CBD due to its stability and capacity to mobilize eCBs and facilitate their anti-inflammatory actions.

BAK+ELN also repressed expression of genes linked to KC differentiation but upregulated those associated with increasing the stemness of cells. Finally, BAK+ELN inhibited cortisol secretion in RHE skin (not observed with CBD). These results support a model in which BAK and ELN synergistically interact to inhibit eCB degradation (Figure 1), favoring eCB mobilization and inhibition of downstream inflammatory mediators (e.g., TNF, COX-2, type I interferon) [12].

These findings define novel physiological functions for Bakuchiol and provides a rationale for understanding its distinct biological activities. Topical combination of these ingredients may thus enhance cutaneous eCB tone or potentiate other modulators, which may be beneficial for skin

conditions such as acne and atopic dermatitis.

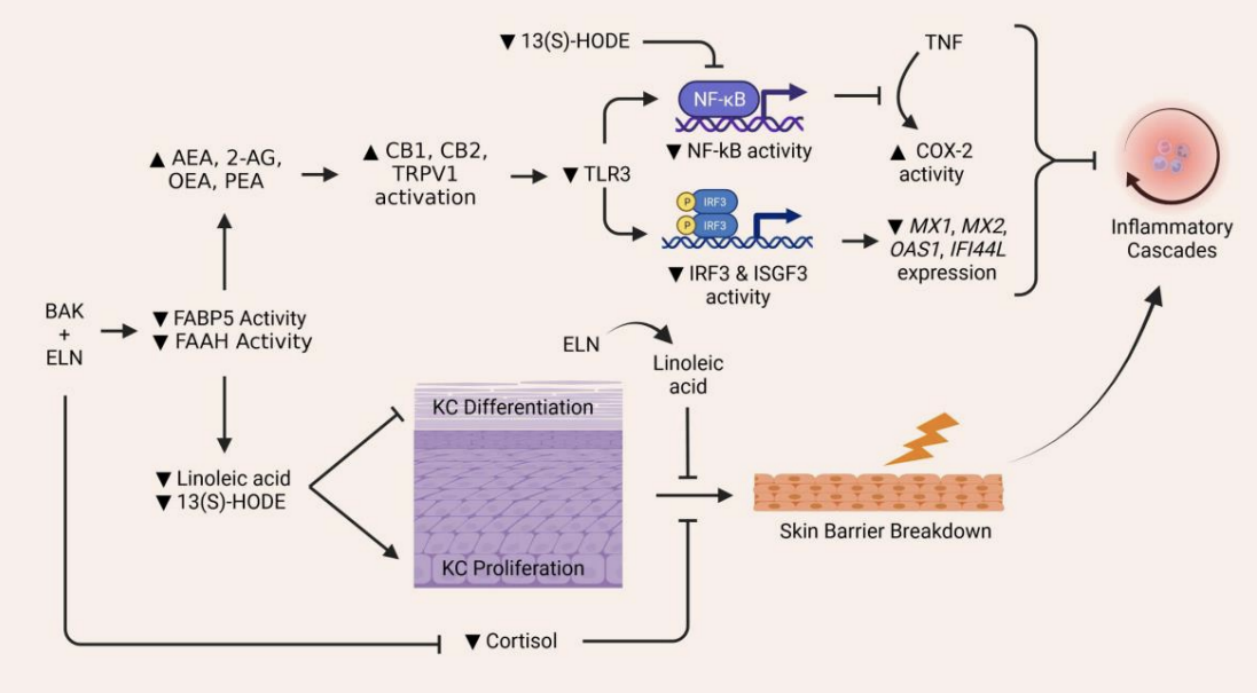

Figure 1. Bakuchiol (BAK)+Ethyl Linoleate/Oleate (ELN) hypothesized mechanisms of action.

BAK+ELN inhibits FABP5 and FAAH, resulting in increased abundance of eCB ligands (AEA, 2-AG, OEA, PEA). Ligands interact with receptors (CB1, CB2, TRPV1) with downstream anti-inflammatory effects. Such effects are mediated by decreased TLR3 expression with inhibition of transcription factors (e.g., NF-κB, IRF3, ISGF3). Loss of NF-κB activity blocks activation of COX-2 by TNF while loss of IRF3 and ISGF3 DNA binding decreases type I interferon gene expression, leading to decreased inflammatory cell activation. Loss of FABP5 and FAAH activity favors a proliferative KC phenotype, mediated by decreased abundance of linoleic acid and 13(S)-HODE. The risk of skin barrier compromise is countered by conversion of ELN to linoleic acid and loss of cortisol production. (Figure created using BioRender).

Conclusions

The importance of the ECS in epidermal homeostasis has become clear in recent decades,

suggesting directions for development of topical therapies based upon cutaneous eCB regulation [1]. This study showed that CBD only weakly inhibited eCB degradation mediators and cytokine pathways. In contrast, BAK and ELN in combination potently repressed both FABP5 and FAAH and had anti-inflammatory effects not seen in KCs treated with CBD or with BAK or ELN separately. The study demonstrates new approaches to modulating eCB tone in skin and demonstrate synergy between Bakuchiol and Ethyl Linoleate/Oleate, two well-known safe and effective natural products, which can be exploited for development of a wide array of skin treatment products. Such products could be beneficial for compromised skin conditions such as ichthyosis, acne, or atopic dermatitis.

References

1. G Martinelli et al., Cannabis sativa and skin health: Dissecting the role of phytocannabinoids, Planta Medica, 2022; 88:492-506

2. M Pucci et al., Endocannabinoid signaling and epidermal differentiation, European J Dermatology, 2011; 21 Suppl 2(2):29-34.

3. LE Filipiuc et al., Major Phytocannabinoids and their related compounds: should we only search for drugs that act on cannabinoid receptors? Pharmaceutics 2021;13(11),

4. W Jaidee et al. Kinetics of CBD, Δ(9)-THC degradation and cannabinol formation in Cannabis Resin at various temperature and pH conditions. Cannabis and Cannabinoid Research, 2022; 7(4):537- 547.

5. Y Gaoni and RJT Mechoulam, Hashish—VII: The isomerization of cannabidiol to tetrahydrocannabinols, Tetrahedron, 1966;22(4):1481-1488; P Golombek et al., Conversion of cannabidiol (CBD) into psychotropic cannabinoids including tetrahydrocannabinol (THC): A controversy in the scientific literature, Toxics, 2020; 8(2): 41.

6. WR Swindell et al., Psoriasis drug development and GWAS interpretation through in silico analysis of transcription factor binding sites, Clinical and Translational Medicine, 2015;4:13. 7. WR Swindell et al., Modulation of epidermal transcription circuits in psoriasis: new links between inflammation and hyperproliferation. PloS one, 2013;8(11):e79253.

8. T Biro et al., The endocannabinoid system of the skin in health and disease: novel perspectives and therapeutic opportunities. Trends Pharmacol Sci, 2009;30(8): 411–420.

9. JL Napoli, Cellular retinoid binding-proteins, CRBP, CRABP, FABP5: effects on retinoid metabolism, function and related diseases, Pharmacol Therapy, 2017; 173:19–33 10. RK Chaudhuri & K Bojanowski, Bakuchiol: a retinol-like functional compound revealed by gene expression profiling and clinically proven to have anti-aging effects, International J Cosmetic Science, 2014;36(3):221-30.

11. RK Chaudhuri, Bakuchiol: A retinol-like functional compound, modulating multiple retinol and non-retinol targets. In Cosmeceutical & Active Cosmetics, Third Edition, Eds, RK Sivamani, J Jagdeo, P Elsner & HI Maibach, CRC Press, 2015:1-18.
12. Swindell et al., Bakuchiol and ethyl (Linoleate/Oleate) synergistically modulate endocannabinoid tone in keratinocytes and repress inflammatory pathway mRNAs, J Investigative Dermatology Innovation, 2022, Accepted for publication.