Cannabinoids as Therapeutics for Non-Melanoma and Melanoma Skin Cancer

Soliman E1, Ladin DA1, and Rukiyah Van Dross1,2*

1Department of Pharmacology and Toxicology, East Carolina University, USA
2Center for Health Disparities, East Carolina University, USA

Abstract

Skin cancer has a significant impact on the health care system of many societies. The most common types of skin cancer are Non-Melanoma Skin Cancer (NMSC) and melanoma. In the United States, NMSC incidence is greater than all other cancers combined. Although death is a rare outcome of NMSC, recurrence is fairly common. In contrast, melanoma is highly metastatic, chemoresistant and is often lethal. As such, novel therapeutics are urgently needed for treatment of these malignancies. The therapeutic potential of cannabinoids has been investigated for numerous conditions including skin cancers. The actions of cannabinoids are regulated by components of the endocannabinoid system (ECS) which include cannabinoid receptors, putative transporters, and enzymes involved in endocannabinoid synthesis and degradation. Recent evidence suggests that cannabinoids of endogenous origin perform specific roles in skin cell homeostasis and in the development and elimination of skin cancer. Hence, synthetic cannabinoids (psychoactive and non-psychoactive), phytocannabinoids and endocannabinoids are being investigated as potential therapeutics for skin cancer. In this article, current knowledge regarding the role cannabinoids in skin cancer development and the potential for utilizing cannabinoids as novel therapeutics are reviewed.

ABBREVIATIONS

2-AG: 2-Arachidonoyl Glycerol; ABDH: Alpha/Beta-Hydrolase Domain; AEA: Arachidonoyl Ethanolamide; BBB: Blood-Brain-Barrier; CB1: Cannabinoid Receptor Type 1; CB2: Cannabinoid Receptor Type 2; CNS: Central Nervous System; COX-2: Cyclooxygenase-2; DMBA: Dimethylbenz [a]-Anthracene; ECS: Endocannabinoid System; EMT: Endocannabinoid Membrane Transporter; FAAH: Fatty Acid Amide Hydrolase; MAGL: Monoacylglycerol Lipase; NMSC: Non-Melanoma Skin Cancer; P-450: Cytochrome P-450; ∆9-THC: ∆9-Tetrahydrocannabinol; TPA: 12-O-Tetradecanoylphorbol-13-Aacetate; TRPV1: Transient Potential Vanilloid Receptor; UVB: Ultraviolet Light B.

INTRODUCTION

The endocannabinoid system

Cannabinoids are utilized therapeutically to prevent pain and chemotherapy-induced emesis. More recently, cannabinoids have been identified as potential therapeutics for conditions including epilepsy, autoimmune disease and cancer [1]. Cannabinoids are a class of structurally diverse hydrophobic compounds that are derived from animals (endocannabinoids, e.g., arachidonyl ethanolamide (AEA) and 2-arachidonyl glycerol (2-AG)), plants (phytocannabinoids, e.g, ∆9-THC: ∆9-Tetrahydrocannabinol), or they are chemically synthesized (e.g., WIN 55,212-2 and JWH-015). Cannabinoids activate G-protein-coupled, cannabinoid receptor type 1 (CB1) or cannabinoid receptor type 2 (CB2) which then inhibits cAMP production and decreases intracellular calcium accumulation [2,3]. Other cannabinoid responsive receptors have also been identified including the transient receptor potential cation channel, subfamily V1 (TRPV1) and the orphan G-protein coupled receptor, GRP55 [4,5]. Numerous reports show that cannabinoids can also produce cellular effects independent of the cannabinoid receptors [6,7]. Endocannabinoids enter cells through endocannabinoid membrane transporters (EMT), although the existence of this transporter remains controversial [8]. Once inside cells, the activity of endocannabinoids is terminated by fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) or alpha/beta hydrolase domain (ABHD) family proteins [9-11]. Other enzymes including cycooxygenases, lipoxygenases, and P-450 enzymes also convert endocannabinoids to signaling lipids that possess distinct cellular targets and biological activity [12]. The endocannabinoids, cannabinoid receptors, molecular transporters and enzymes involved in cannabinoid synthesis and metabolism are collectively referred to as the endocannabinoid system (ECS). The ECS is widely distributed and has been most extensively characterized in the central nervous system (CNS) and the immune system. Activation of the ECS in the CNS impacts...
various processes such as learning and memory, reward and motivation and the appetite primarily through CB1-mediated signaling [13]. Alternatively, the peripheral ECS modulates the immune system, lipid metabolism and other biological activities through both CB1 and CB2 receptors [14].

Unlike neural and immunologic tissues, the physiological function of the ECS in the epidermis is poorly understood. The epidermis is comprised of several cell types including keratinocytes and melanocytes, which are the source of non-melanoma skin cancer (NMSC) and melanoma, respectively [15]. Components of the ECS were found to be present and functional in normal human and animal skin cells. It has been proposed that AEA, the EMT, FAAH and cannabinoid receptors are involved in maintaining skin homeostasis by modulating proliferation and differentiation [16-18]. The cannabinoid receptors are also expressed in neoplastic tissues including NMSC and melanoma [17,19]. Furthermore, exogenous administration of endocannabinoids, phytocannabinoids or synthetic cannabinoids to NMSC or melanoma cells reduces its proliferation and induces apoptosis in vitro and in vivo [16,17,20]. These collective findings indicate that the ECS is involved in carcinogenic processes and its elements may therefore be appropriate targets for novel skin cancer therapeutics [21,22]. The goal of this review was to examine the literature to gain a greater understanding of the effects of the ECS on skin cancer development and to assess the feasibility of utilizing cannabinoids as novel therapeutics against NMSC and melanoma.

Non melanoma skin cancer (NMSC)

The most common types of NMSC include basal cell carcinoma and squamous cell carcinoma. There are approximately 5.4 million new cases diagnosed each year [23] making NMSC the most frequently acquired cancer in the United States. NMSCs typically appear on sun-exposed areas of the skin such as the face, lips, ears, neck, and backs of hands and neck (basal cell carcinoma) as well as the head and neck (basal cell carcinoma) [24]. The major risk factor for developing NMSC is excessive exposure to natural (solar) and artificial (tanning beds) ultraviolet (UV) light as well as contact with chemical carcinogens such as arsenic and the polycyclic aromatic hydrocarbons [25]. Since the incidence of NMSC is continually rising due to increased exposure to UV radiation, novel treatments for NMSC are needed.

Role of the ECS in NMSC Development and Treatment

An accumulating body of evidence implicates the ECS in NMSC development. It has been reported that CB1 and CB2 receptors were expressed in a number of human and murine transformed epidermal cell lines as well as in skin tumors (e.g. papilloma, basal cell carcinoma, squamous cell carcinoma) [17]. Furthermore, the expression of orphan receptor, GPR55 was higher in human squamous cell carcinoma samples than in normal skin [26]. NMSC is typically studied using cultured transformed keratinocytes and by utilizing two well-known animal models, the UVB-light protocol [27] or the two-stage chemical carcinogenesis protocol with dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) [28]. Using the UVB-light tumorigenesis model, Zheng et al., demonstrated that CB1 and CB2 receptor deficient mice (CB1/2-/-) developed fewer papillomas than the corresponding wild-type (CB1/2+/-) animals under conditions where UVB light increased the activity of both receptor types [29]. Moreover, acute exposure of CB1/2+/- but not CB1/2-/- animals to UVB resulted in elevated inflammation consistent with the known role of inflammation in cancer promotion [29]. However, a different study showed that the synthesis of AEA and 2-AG and the cell membrane localization of CB1 and CB2 were reduced after UVB exposure indicating that the ECS might be protective against tumor development [30]. In contrast to studies conducted with UVB-light, CB1 and CB2 receptors did not modulate tumor development initiated by DMBA/TPA. Both CB1/2+/- and CB1/2-/- mice developed papillomas with no significant difference in tumor multiplicity or incidence [31]. However, GPR55 receptors were required for DMBA/TPA-induced skin carcinogenesis. Specifically, it was determined that GPR55+/+ mice were more resistant to papilloma formation than wild type mice [26]. Thus, the available data suggests that the involvement of the cannabinoid receptors in tumor development is stimulus-dependent.

The potential therapeutic effect of synthetic cannabinoids on NMSC has been examined. Synthetic cannabinoid, WIN-55 212–2 (mixed CB1/CB2 agonist) and the non-psychotropic synthetic, JWH-133 (selective CB2 agonist) reduced the growth of murine epidermal tumor cells in PDV.C57 xenograft experiments [17]. In this model, impairment in tumor vascularization and a reduction in the expression of various proangiogenic factors were also observed. Complementary in vitro studies demonstrated that WIN-55 212–2 and JWH-133, increased DNA fragmentation and death in PDV.C57 cells which was reversed by selective CB1 and CB2 receptor antagonists [17]. More recently, the chemopreventive activity of other synthetic cannabinoids was tested in the DMBA/TPA skin carcinogenesis model [32]. Topical application of JWH-018, JWH-122, and JWH-210 (mixed CB1/CB2 agonists), thirty minutes before each TPA administration, resulted in a significant reduction in tumor incidence and multiplicity compared to vehicle treated animals.

Other studies show that the endocannabinoid, AEA, effectively eliminates NMSC cells. NMSC over expresses the enzyme, cyclooxygenase-2 (COX-2), differentiating it from normal keratinocytes. It was determined that AEA was metabolized by COX-2 to a novel metabolite, 15deoxy, Δ12,14 prostaglandin-ethanolamide J2 (15d-PGJ2-EA) whose production was required for AEA cell death [33]. AEA-induced apoptosis in NMSC cells was mediated by the initiation of oxidative stress and endoplasmic reticulum (ER) stress. Furthermore, inhibition of AEA degradation with the FAAH inhibitor, URB597, increased J-series prostaglandin synthesis and AEA cytotoxicity [34]. However, selective antagonist of CB1, CB2, and TRPV1 receptors did not inhibit AEA-induced ER stress and apoptosis [7]. Since the endogenous levels of COX-2 are low in non-tumorigenic keratinocytes compared to tumorigenic keratinocytes, the data suggest that AEA is selectively toxic towards tumor cells.

The results of these reports suggest that cannabinoid receptor stimulation by chemical carcinogens and UVB light promotes tumor development but that exogenously administered cannabinoid ligands induce tumor cell death. These seemingly opposing effects are possibly explained by the concentration-
dependent effects of cannabinoids on skin cancer cell viability. Whereas the endogenous levels of endocannabinoids (nanomolar range) associated with carcinogen exposure increases tumor growth, the exogenous administration of cannabinoids (micromolar range) decreases tumor growth. This suggestion is consistent with our previous studies demonstrating that low concentrations of anandamide (< 10 µM) increased NMSC cell proliferation while high concentrations (> 10 µM) caused cell death and apoptosis [35]. Collectively, the data suggest that ECS components are functional in keratinocytes and can be targeted to bring about tumor cell death.

**Melanoma**

Melanoma is the most aggressive and deadly cutaneous neoplasm in the United States. It is estimated that more than 76,000 new cases of melanoma will be diagnosed and that over 10,000 patients will die from this disease in 2016 [36]. Like NMSC, UV radiation exposure is a primary risk factor for developing melanoma [37]. In addition, individuals with family history, poor immune function or rare genetic abnormalities such as xeroderma pigmentosum may be at increased risk of developing this malignancy [38]. Unlike other forms of skin cancer, melanoma is especially dangerous due to its highly metastatic nature and resistance to chemotherapy [39]. Melanoma incidence has steadily risen over the last 10 years and novel therapeutic approaches must be identified.

**Role of the ECS in melanoma development and treatment**

Recent research suggests that the ECS plays a critical role in melanoma development and progression. Cannabinoid receptors CB1 and CB2 are expressed in human melanomas and numerous melanoma cell lines suggesting functional endocannabinoid signaling in this cancer type [19]. Indeed, plasma concentrations of 2-AG were elevated in patients with metastatic melanoma suggesting a link between endocannabinoid regulation and melanoma development [40]. The data also indicates that endocannabinoids act in a concentration-dependent manner. At low levels (<10 µM), endocannabinoids released from tumor cells including melanoma, act on immune cell cannabinoid receptors, leading to immune suppression and tumor cell mediated immune evasion [41-43]. High endocannabinoid levels (>10 µM) in the tumor microenvironment are suggested to suppress tumor growth and migration [44,45]. As such, the therapeutic potential of cannabinoids in melanoma skin cancer has been investigated.

Melanoma is typically studied using cultured melanoma cells or subcutaneous tumor models. Using these experimental approaches, several reports indicate that phytocannabinoids are potent inducers of melanoma cell death. Armstrong et al. demonstrated that Δ⁹-THC induced apoptosis in melanoma cell lines including A357, SK-MEL-28, and CHL-1 through an autophagic-dependent mechanism [46]. In this study, the cytotoxicity of Δ⁹-THC was further increased in melanoma cells co-treated with cannabidiol (CBD). Furthermore, the anti-tumor effects were replicated in a melanoma xenograft mouse model [46]. Similarly, Glodde’s group reported that Δ⁹-THC decreased the growth of melanoma in vitro and in vivo and that this effect was not observed in mice lacking CB1 and CB2 receptors, pointing to a CB receptor-dependent mechanism [31]. Interestingly, a different report demonstrated that Δ⁹-THC preferentially eliminated melanoma compared to non-tumorigenic melanocytes by inhibiting the activity of the pro-survival proteins, Akt and pRb [19]. As such, phytocannabinoids may emerge as a selective and effective class of anti-melanoma agents.

Synthetic cannabinoids have helped to reveal the role CB1 and CB2 receptors in melanoma and the potential of CB receptors as therapeutic targets. Activation of CB1/CB2 with WIN55,212-2 decreased melanoma cell viability, melanoma xenograft growth and metastasis [19,47]. In contrast, CB2 receptor activation with JWH-133 had no effect on melanoma cell viability but decreased melanoma cell migration across the blood brain barrier suggesting specific roles for CB1 and CB2 in melanoma metastasis [48,49]. The synthetic, metabolically stable analogs of AEA such as arachidonyl-2’-chloroethylamide (ACEA) and anandamide (m-AEA) have also been examined for their anti-melanoma properties. In contrast to other synthetic cannabinoids, these agents possessed moderate toxicity in human melanoma cell lines but failed to significantly decrease tumor size in mouse melanoma xenografts [50].

The endocannabinoid AEA shows promise as a potential anti-melanoma agent. Consistent with observations in NMSC, AEA appears to promote tumor death through a receptor independent pathway [50,51]. Adinolfi et al. showed that AEA caused melanoma cell death that was partially reversed by pretreatment with a CB1 antagonist [48]. Furthermore, the FAAH inhibitor, URB597, potentiated the anti-melanoma properties of AEA and inhibition of COX-2 significantly reversed AEA-mediated cytotoxicity [48]. These findings suggest that COX-2 metabolized AEA to cytotoxic prostaglandin-ethanolamides in melanoma as well as NMSC. In the same study, disruption of cellular lipid rafts reversed the effect of AEA on melanoma cell viability suggesting that these organization centers were important for the activity of AEA [48]. Taken together, these reports indicate that endocannabinoids may induce tumor cell toxicity through mechanisms distinct from other classes of cannabinoid compounds.

**CONCLUSION**

An assessment of the available findings indicates that components of the ECS play an important role in the development of NMSC and melanoma which have a higher incidence than all other cancers combined. The ECS is involved in the formation of UVB-induced NMSC and melanoma.

Endocannabinoids, synthetic cannabinoids and phytocannabinoids decrease NMSC and melanoma growth in vitro and in vivo through CB receptor dependent and independent pathways (Table 1). This suggests that molecules which make up the ECS are potential targets for development of novel skin cancer therapeutics. Because NMSC and melanoma rates are rapidly increasing, new therapeutic options are needed. The studies cited herein indicate that a more detailed assessment of the effect of cannabinoids on skin cancer is warranted and that agents which

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**REFERENCES**

1. Armstrong et al. (2016) demonstrated that Δ⁹-THC are potent inducers of melanoma cell death.

2. Van Dross et al. (2016) and other studies indicate that endocannabinoids preferentially eliminate melanoma compared to non-tumorigenic melanocytes by inhibiting the activity of the pro-survival proteins, Akt and pRb.

3. Melanoma incidence has steadily risen over the last 10 years and novel therapeutic approaches must be identified.

4. Δ⁹-THC induces apoptosis in melanoma cell lines including A357, SK-MEL-28, and CHL-1 through an autophagic-dependent mechanism.

5. Δ⁹-THC was further increased in melanoma cells co-treated with cannabidiol (CBD).

6. Δ⁹-THC decreased the growth of melanoma in vitro and in vivo.
### Table 1: Anticancer activity of cannabinoids.

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modulate ECS components may be effective in clinical settings.

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