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Review Article

Cannabinoid system in the skin – a possible target for future therapies in dermatology

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Abstract: Cannabinoids and their derivatives are group of more than 60 biologically active chemical agents, which have been used in natural medicine for centuries. The major agent of exogenous cannabinoids is Δ^9-tetrahydrocannabinol (Δ^9-THC), natural psychoactive ingredient of marijuana. However, psychoactive properties of these substances limited their use as approved medicines. Recent discoveries of endogenous cannabinoids (e.g. arachidonoylethanolamide, 2-arachidonoylglycerol or palmithyloethanolamide) and their receptors initiated discussion on the role of cannabinoid system in physiological conditions as well as in various diseases. Based on the current knowledge, it could be stated that cannabinoids are important mediators in the skin, however their role have not been well elucidated yet. In our review, we summarized the current knowledge about the significant role of the cannabinoid system in the cutaneous physiology and pathology, pointing out possible future therapeutic targets.

Key words: cannabinoid receptors – drug development – endocannabinoids – keratinocytes – skin

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Introduction

Narcotic and therapeutic properties of cannabis derivates have been known for centuries, and marijuana has remained as one of the most widely used drugs worldwide (1). However, the exact mechanism of marijuana action was unknown until the discovery of cannabinoid receptors (CBRs) at the end of twentieth century. The identification of endogenous ligands of CBRs (so called endocannabinoids) initiated a rapid progress in the understanding of the role of the cannabinoid system in physiological and pathological processes in human beings. Recently, CBRs have also been demonstrated to be expressed in healthy and diseased skin (2), suggesting that the alteration of the cannabinoid system could be important for the development of numerous skin diseases. Therefore, we performed a review of available literature data to summarize current knowledge about the cannabinoid system in the skin pathophysiology pointing out possible future therapy targets.

Abbreviations: AEA, arachidonoylethanolamide (anandamide); 2-AG, 2-arachidonoylglycerol; AMT, AEA membrane transporter; cAMP, cyclic adenosine monophosphate; CBRs, cannabinoid receptors; CB_1R, cannabinoid receptor 1; CB_2R, cannabinoid receptor 2; EGF-R, epidermal growth factor receptor; FAAH, fatty acid amide hydrolase; CGRP, calcitonine gene-related peptide; GPR, G-protein-coupled receptor; HaCaT, spontaneously immortalized human keratinocytes; HMVEC, human dermal microvascular endothelial cells; KSHV, Kaposi's sarcoma-associated herpes virus; MAP kinase, mitogen-activated protein kinase; NHEK, normal human epidermal keratinocytes; PEA, N-palmithylethanolamide; PKA, protein kinase A; PPAR, peroxisome-proliferators-activated receptor; Δ^9-THC, Δ^9-tetrahydrocannabinol; TRPV-1, transient receptor potential vanilloid-1.

Endocannabinoids receptors

Two receptor types for endocannabinoids have been identified till now beyond all doubts: CB_1R (cannabinoid receptor 1) and CB_2R (cannabinoid receptor 2) (3). Both receptors belong to the large superfamily of G-protein-coupled receptors (GPRs) with the primary structure characterized by seven hydrophobic α-transmembrane domains (each consisting of about 20–25 amino acids), which are connected by alternating intra- and extra-cellular loops. A typical feature of GPRs, which is also found in CBRs, is their ability to form intramolecular disulfide bridges between cysteins in the second and third domain, which stabilize a tertiary structure of receptors (3). Another

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common characteristic is a highly conservative fragment without proline in the fifth hydrophobic domain. In addition, extra-cellular N-terminus contains three sites of glycosylation, but their function is still to be elucidated (3–7).

Cannabinoid receptor 1 is predominantly expressed in central nervous system and in tissues and cells of immune system. CB_2R has been found mainly in non-neuronal tissues (5,8–10). CB_1R and CB_2R have been characterized and cloned from mammalian tissues at the beginning of 1990s (6). The mRNA sequences encoding CB_1R and CB_2R have been defined for vertebrates including human, mouse, rat, cat, cow, newt, puffer fish and zebra fish, as well as for non-vertebrate such as leech. The amino acid sequence of CBR subfamily is a conservative one. The human amino acid sequence of CB_1R and CB_2R is identical in more than 40% (1,11).

Interestingly, recent trials on endocannabinoids indicated the possibility of existence of CB_1R/CB_2R-independent mechanisms of action. Experiments with double $CB_1R^{-/-}$ and $CB_2R^{-/-}$ knockout mouse suggested the presence of the CB_3 receptor, which still has to be cloned and characterized (12). Moreover, it was shown that receptors belonging to peroxisome-proliferators-activated receptor (PPAR) family as well as a transient receptor potential vanilloid-1 (TRPV-1) could be also activated by cannabinoids (see below) (3,4,12,13). Remarkably, recently some authors even observed that endocannabinoids can simultaneously activate various receptors on the same cell [e.g. CB_1R and TRPV-1 (14) or CB1R, TRPV1 and PPAR-γ (15)], and only interaction with all these receptors produced the full action of endocannabinoids.

Cannabinoid receptor type 1 (CB_1R)

The gene encoding CB_1R is localized on chromosome 6 (6q14-q15) (16). The CB_1R gene is intronless and very conservative one with similar sequence in humans, rats and mice (3,17). Its mRNA has been detected in embryonic mouse, as soon as at 11th day of mouse gestation. Postnatal expression of this receptor has mainly been detected in the brain and spinal cord (18). The highest density of CB_1R has been showed in basal ganglia, substantia nigra, pars reticulata, globus pallidus, hippocampus, particularly within the dentate gyrus, as well as in the molecular layer of the cerebellum (19). The expression of CB_1R in central nervous system correlates with the level of γ-aminobutyric acid and glutamate-gated ion channels (20). CB_1R has been demonstrated to be localized presynaptically on GABA-ergic and glutamatergic interneurons (21), which may indicate a role of CB_1R in neuromodulation of signal transmission (7,17,22). Two splice variants of CB_1R have been identified: $CB_{1A}R$ with altered terminal sequence and $CB_{1B}R$ with deletion of 33 amino acid sequence in N-terminus, but their role is still not known (23). CB_1R expression have been detected not only in central nervous system, but also in peripheral organs, including heart, lungs, gastrointestinal tract, liver, adrenal glands, bladder, placenta, uterus, ovaries, testes, spermatic duct, skin and adipose tissue (19,24,25).

Cannabinoid receptor type 2 (CB_2 R)

The CB_2R gene is localized on chromosome 1 (1p36,11) (26). CB_2R mRNA has been detected during both, pre and postnatal live (18). CB_2R expression is typical for tissues associated with immune system and, for that reason, this receptor has been called as *immunocannabinoid system receptor* (7,8,17,27). CB_2R has been detected on B and T lymphocytes, NK cells, monocytes and in immune organs such as spleen, tonsils and thymus (8). Although recent studies suggested its presence in the brain and on peripheral nerves, it must be mentioned that regarding central nervous system, till now CB_2R in this location was mainly documented in neoplasms (28).

Non-CB_1/CB_2 receptors activated by cannabinoids

It was observed that cannabinoids can activate numerous other receptors. GPR-55 and GPR-119 are two putative co-receptors, which could interact with cannabinoid ligands and activate non-CB_1R/CB_2R mechanisms. GPR-55 has been reported to be activated by various cannabinoids, while GPR-119 is a receptor for oleoylethanolamide. The role of these receptors is not known. Data originating from the study on transgenic GPR-$55^{-/-}$ mouse indicated that GPR-55 could be important in cardiovascular system, inflammation and pain (29,30).

Another target for cannabinoids could be the TRPV-1, a ligand-gated, non-selective ion channel (31,32). Expression of TRPV-1 has been affirmed in some types of central neurons, perivascular sensory nerves, immune cells such as macrophages, dendritic or Langerhans cells, endothelial and epithelial cells, epidermal and hair follicle keratinocytes as well as in smooth muscle cells (33,34). TRPV-1 can be activated by numerous inflammatory mediators and chemicals including capsaicin and endocannabinoids (14). Activation of TRPV-1 is regulated by phosphokinases, such as protein kinase A (PKA), protein kinase C and calcium/calmodulin dependent kinase IIα; dephosphorylation, and deactivation of this receptor starts with activation of protein phosphatase 2B (calcineurin) (35). Activation of TRPV-1 by anandamide induces vasodilatation, calcitonine gene-related peptide (CGRP) release and nitric oxide (NO) synthesis, inhibits L-type calcium channels and intracellular calcium mobilization as well as decreases production of cyclic adenosine monophosphate (cAMP) (14,36,37). It was shown that CB_1R and TRPV-1 are co-localized on sensory neurons in the skin (38).

Peroxisome-proliferators-activated receptors are the next putative collaborators in cannabinoid system. PPARs play

important role in regulation of lipid metabolism, hepatic peroxisomal enzyme expression, insulin sensitivity, glucose metabolism and inflammation (39,40). Natural PPAR agonists include fatty acids and eicosanoid derivates (39,40). Recently, endocannabinoids have also been found to directly activate PPAR-α and PPAR-γ (40–42).

Although all these non-CB_1/CB_2 receptors could be activated by cannabinoids, it seems that cannabinoids are not the major group of their ligands. Therefore, in the next paragraphs, we have mostly been concentrated on two major CBRs: CB_1R and CB_2R.

Signal transduction via CBRs

Signal transduction via CBRs is based on G-protein complex. G-proteins belong to a big family of signalling molecules consisting of three subunits (α, β and γ) and possessing GTP-ase activity. CB_1R is coupled with G_o and G_i, whereas CB_2R only with G_o protein. The signal transduction via CB_1R and CB_2R can be inhibited by bacterial toxins: cholera toxin or pertussis toxin that induces covalent ADP-rybosylation of specific G-protein α-subunits of G_i family (43).

The signal transduction in immunocannabinoid system is not completely clear, as it depends on the cell type studied. Figure 1 depicted the probable mode of endocannabinoid action in immune cells, mainly T lymphocytes, upon CBR stimulation based on available literature data. It is generally accepted that activation of CBRs induces exchange of GDP to GTP in α subunit and subsequent dissociation of α and $\beta\gamma$ subunits (12,17,44). This leads to the inhibition of adenylate cyclase that results in reduction in intracellular cAMP level; however, the magnitude of this effect could be dependent on particular cellular isoform of adenylate cyclase (7,17). Diminished cAMP level intracellularly suppresses activity of PKA and induces changes in ion distribution via interaction of dissociated $\beta\gamma$ subunit with respective ion channels leading to increased cytosolic calcium ion concentration (45,46). As a final consequence, translocation of critical transcriptional factors such as NF-AT, NF-κB, CREB/ATF into nucleus is inhibited that change the gene expression of a number of interleukins, chemokines and growth factors, e.g. interleukin 2, interleukin 8 or interferon γ (47,48) (Fig. 1).

Changes in calcium ion distribution upon CBR stimulation may also activate phospholipase C, which via secondary messengers lead to activation of the family of multifunctional mitogen-activated protein (MAP) kinases, such as p44/42 MAP kinase, JUN-terminal kinase and p38 MAP kinase (7,17). Finally, this enables the action of AP-1 transcriptional factor (Fig. 1).

It seems probable that individual elements of cannabinoid signal transduction pathway in immune cells may be more or less pronounced in various physiological and pathological situations depending upon co-stimulatory effect of other signals that are received by cells.

Ligands of CBRs

Cannabinoids are a group of more than 60 biologically active chemical agents which are synthesized by animals (endocannabinoids), produced by plants (e.g. *Cannabis sativa*) (phytocannabinoids) or developed artificially in laboratories (synthetic cannabinoids) (1–4,49).

Arachidonoylethanolamide (anandamide or AEA), 2-arachidonoylglycerol (2-AG), virodhamine, N-arachidonoyldopamine, arachidonyl-2′-chloroethylamide or N-palmitoylethanolamide (PEA) represent a group of endocannabinoids that include amides or esters of long chain polyunsaturated fatty acids (Fig. 2) (50–54). Generally, they have been categorized to neuromodulatory agents, but they have some peculiar features distinguishing them from typical neurotransmiters. They are synthesized in place of their action upon demand by receptor-stimulated cleavage of membrane lipid precursors and are not preserved in synaptic vesicles. Endothelial cells and resident macrophages are probably main source of AEA outside the central nervous system (55). Lipophilic nature of endocannabinoids allows them to activate enzymes in cytosol and transmembrane compartments, where they can interact with lipoprotein structures (5,7,17,18).

Phytocannabinoids are group of agents similar to terpenophenols with lipophilic properties. The major exogenous cannabinoid is Δ^9-tetrahydrocannabinol (Δ^9-THC), a natural psychoactive ingredient of marijuana (49). Most synthetic cannabinoids are derivatives of Δ^9-THC. CP-55940, frequently labelled with tritium ([^{3}H]-CP-55940), and WIN-55,212-2 belong to the most known representatives of synthetic CBR agonists and have been used to detect CB_1R and CB_2R expression (49,56,57).

Biosynthesis and regulation of endocannabinoids

Synthesis of endocannabinoids is controlled by a specific class of enzymes that maintain physiological levels of this molecule. AEA, the most extensively studied endocannabinoid, is synthesized in a two-step enzymatic pathway: the first step of AEA synthesis involves calcium-dependent transacyclase, which catalyses formation of N-acyl phosphatidylethanolamines from phosphatidylocholine and phosphatidylethanoloamine followed by hydrolysis by phospholipase D to AEA and related fatty acid amides (58,59). The level of AEA is controlled by AEA membrane transporter (AMT) that removes AEA from extra-cellular space and fatty acid amide hydrolase (FAAH), which participates

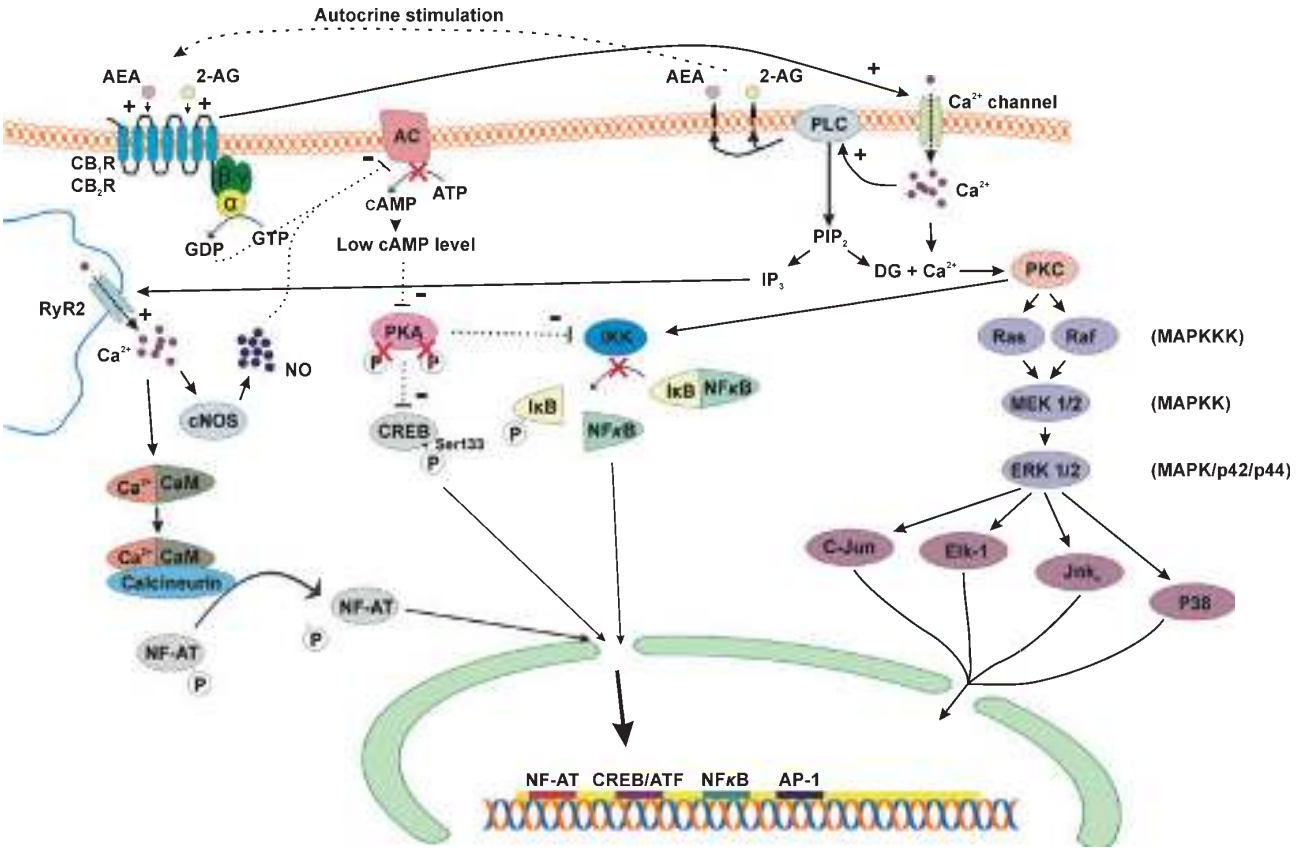

Figure 1. The theoretical model of signal transduction via cannabinoid receptors in T-cells: activation of CBRs induces exchange of GDP to GTP in α subunit and subsequent dissociation of α and $\beta\gamma$ subunits leading to inhibition of adenylate cyclase that results in reduction of intracellular cAMP level. Diminished cAMP level intracellularly suppresses activity of PKA and induces changes in ion distribution via interaction of dissociated $\beta\gamma$ subunit with respective ion channels leading to increased cytosolic calcium ion concentration. As a final consequence, translocation of critical transcriptional factors such as NF-AT, NF-κB, CREB/ATF into nucleus is inhibited. Changes in calcium ion distribution upon CBR stimulation also activate phospholipase C, that via secondary messengers lead to activation of the family of multifunctional mitogen-activated protein (MAP) kinases, such as p44/42 MAP kinase, JUN-terminal kinase and p38 MAP kinase (7,17). Finally, this enables the action of AP-1 transcriptional factor. (AEA, anandamide; 2-AG, 2-arachidonoylglycerol; ATP, adenosine triphosphate; Ca^{2+}, calcium ionsl; CaM, calmodulin; cAMP, cyclic adenosine monophosphate; CB_1R, cannabinoid receptor 1; CB_2R, cannabinoid receptor 2; cNOS, cytoplasmic NO synthase; DG, diacylglycerol; GDP, adenosine diphosphate; GTP, adenosine triphosphate; IκB, cytoplasmatic inhibitor of NFκB; IP_3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; NFκB, nuclear factor κB; NO, nitric oxide; PIP_2, phosphatidylinositol bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C, arrows, stimulatory effect; dotted line, inhibitory effect; red crosses, actions inhibited by cannabinoids).

in intracellular AEA degradation (60,61). The mouse lacking FAAH enzyme show up to 15-fold higher endogenous brain levels of AEA comparing to a wild-type ($FAAH^{+/+}$) mouse (62).

Biosynthesis and enzymatic regulation of 2-AG are still not fully characterized and probably depends on the type of tissues and cells and the type of stimulus (3). One of the most common mentioned pathways for 2-AG synthesis is involvement of phospholipase C and diacylglyccerol lipase that synthesize 2-AG from phospholipid precursors. Monoacylglycerol lipase appears to play the predominant role in 2-AG degradation as a selective blockade of this enzyme produced a number of CB_1R-dependent behavioural effects in mouse including analgesia, hypothermia and hypomotility (63,64).

Besides regulatory effect of enzymes degrading endocannabinoids, their action may be modulated by other mediators as it was observed that bradykinin or prostaglanding E_2 augmented excitatory potency and efficacy of AEA on TRPV-1 in sensory neurons (65).

Cannabinoids in the skin – physiological conditions

The distribution and expression of CB_1R was uniformly found in skin biopsies taken from different body sites (2).

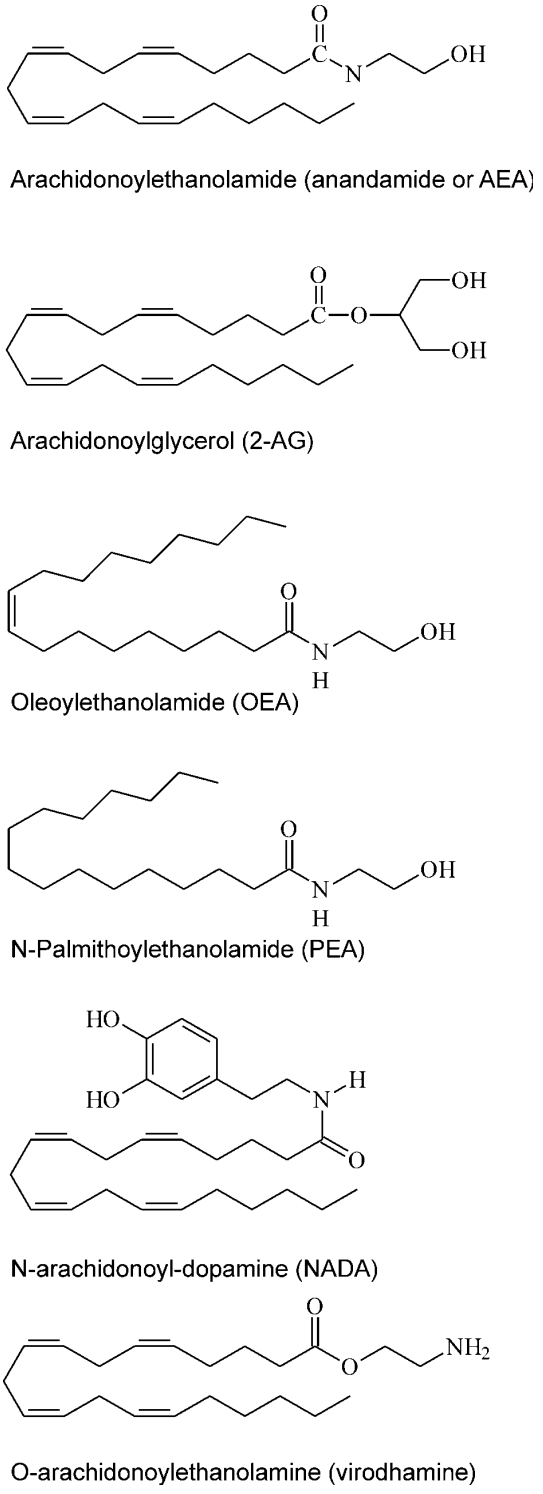

Figure 2. Chemical structures of selected endocannabinoids found in humans.

In the skin, CB_1R was predominantly observed to be expressed on cutaneous nerves (e.g. on large myelinated nerve fibres in the papillary dermis, on small nerve fibres associated with hair follicles and, sporadically, on the nerve fibres in the epidermis) (2). Remarkably, CB_1R-positive sensory nerve fibres also showed co-expression of CGRP (2). In addition, CB_1R immunoreactivity was observed on keratinocytes in the stratum spinosum and stratum granulosum, and on differentiated epithelial cells of infundibulum and the inner hair root sheet in hair follicles (2). CB_1R has also been found on a portion of CD68-positive macrophages and on all dermal mast cells (2). Furthermore, Maccaroni et al. (66) observed that spontaneously immortalized human keratinocytes (HaCaT) and normal human epidermal keratinocytes (NHEK) have the biochemical machinery to synthesize, bind and metabolize AEA, as they observed expression of CB_1R, AMT, FAAH and an AEA-synthesizing N-acyl phosphatidylethanolamine phospholipase D in these cells.

CB_2R has been found in the skin on large myelinated nerve fibre bundles of the superficial and deep reticular dermis, small unmyelinated nerves of the papillary dermis and occasionally on nerves of the epidermis (2). In the epidermis, immunoreactivity for CB_2R has been mainly noted in basal layer. In contrast to CB_1R, CB_2R expression was detected in undifferentiated cells of the infundibulum, in the outer hair root sheet and in the bulb of hair follicle, suggesting that both receptors play different role during differentiation of keratinocytes. Positive immunoreactivity for CB_2R showed also mast cells and CD68-positive macrophages (2). The expression of CB_1R and CB_2R in normal human skin was also reported by other authors (67).

Cannabinoids may exert various effects in the normal skin. It seems that endocannabinoids could be involved in differentiation of keratinocytes. It was observed that in HaCaT and NHEK cells which were stimulated to differentiate exogenous application of AEA inhibited the formation of cornified envelopes, a hallmark of keratinocyte differentiation (66). Interestingly, the activity of AMT and the activity and expression of FAAH increased while the endogenous levels of AEA decreased in HaCaT and NHEK cells that were induced to differentiate *in vitro* (66). It was also shown that AEA downregulates the transcription of genes encoding keratin 1 and 10, transglutaminase 5 and involucrin (68). Other endocannabinoids, including 2-AG, N-arachidonoyl-dopamine and arachidonyl-2′-chloroethylamide, demonstrated similar activity, whereas CB_1R antagonist, SR141716, inhibited the effect of AEA (68). This mechanism seems to be mediated by increasing DNA methylation in human keratinocytes through a p38 MAP kinase, and to a lesser extent p42/44 MAP kinase-dependent pathway triggered by CB_1R (68). Two inhibitors: SB203580 for p38 MAP kinase and PD98059 for p42/44 MAP kinase abolished the effect of AEA on HaCaT cells (68). These observations might suggest that AEA is rather

important in sustaining proliferative phase of cell growth, partaking for instance in early stages of wound healing. We could speculate that blockade of AEA synthesis would promote differentiation of epidermal malignancies. On the other hand, Wilkinson and Williamson (69) found that phytocannabinoids inhibited keratinocyte proliferation in a concentration-dependent manner, although these authors postulated that this phenomenon may be CB_1R/CB_2R independent.

Cannabinoids in immune system

Current evidence about the role of cannabinoids in the regulation of immune system is unquestionable, and even a term 'immunocannabinoid system' has been introduced (8). First reports about the role of cannabinoid system in immune modulations started in the 1970s (70). Expression of both, CB_1R and CB_2R, has been documented in various immune cells and tissues, although it was shown that CB_2Rs exhibited 10- to 100-fold greater reactivity in immune system than CB_1Rs (71). Therefore, CB_2R is thought to be the principal component of immunocannabinoid system (8). The CB_2Rs are expressed by monocytes/macrophages, NK cells, neutrophils and B and T-cells (8,72). Activation of CB_2R usually led to the suppression of immune response (73). *In vitro* studies using mouse cell cultures demonstrated immunosuppressive action of Δ^9-THC, especially on proliferating splenocytes and B-cells but also on macrophages (74). Macrophage function could be regulated by Δ^9-THC on multiple levels, e.g. by down-regulating macrophage-associated cytolysis of tumor cells or decreasing expression of selected proteins released by macrophages which are required for signalling between immune cells (74–76). The cannabinoid system is involved in the regulation of homeostasis between humoral and cellular response (T_{H1} and T_{H2}-dependent) (8,22,77). In experiments with mitogen such as LPS, additional application of AEA or 2-AG suppressed B-cell and splenocyte proliferation response (74). Cannabinoids were also more suppressive for T_{H1} than for T_{H2}-dependent reaction and possessed some anti-inflammatory properties (77). The suppressive effect is mainly directed on activated immune cells (77). It was shown that production of numerous pro-inflammatory cytokines (TNF-α, IL-12, IL-1, IL-6, IL-10) or chemokines (CCL2, CCL5, CXCL8, CXCL10) by activated immune cells could be down-regulated by application of cannabinoids (36,78,79). Immunomodulatory effect of cannabinoids may also be manifested in expression changes of adhesion molecules, such as ICAM-1 or CD62P. Endocannabinoids inhibit T-cell, macrophage and NK-cell activity, as e.g. 2-AG reduced expression of IL-2 gene in murine T-cells, inhibited production of IL-6 in J774 macrophage-like cells and diminished TNF-α synthesis in lypopolisaccharyde stimulated mouse macrophages. Furthermore, endocannabinoids induced migration of human NK and KHYG-1 (a natural killer leukaemia cells) cells (7,77,80) as well as suppressed dose- and time-dependently cytotoxic activity of NK-cells and lymphokine-activated killer cells (70,74). Endocannabinoids also inhibited NO production in macrophages induced by lipopolysaccharide (81). In addition, anandamide-activated lymphocytes showed intensive production of lymphotoxins: different cytokines, eicosanoids, quinolinates and NO (8,74). In another study, a selective activation of CB_2R induced apoptosis of thymocytes *in vitro* and inhibited the proliferative response of T- and B-cells to mitogens through induction of apoptosis (82). This phenomenon involved caspase-8, caspase-9 and caspase-3 activation as well as loss of mitochondrial membrane potential (83). In addition, thymus atrophy, apoptosis and decreased peripheral T-cell response to mitogens was noted *in vivo* (84).

Cannabinoids can modulate IL-2 and TNF-α gene expression as well. Experiments with herpes simplex virus infected mouse revealed a suppressive effect of cannabinoids on IL-2 and TNF-α production. TNF-α secretion was modulated by Δ^9-THC due to inhibition of conversion of pre-TNF-α to an active peptide. Similarly, 2-AG inhibited production of IL-2 in activated T-cells (85). Interestingly, Namazi (86) suggested involvement of cannabinoid system in immune modulation in psoriasis by inhibitory effect on IL-2 and TNF-α release and NO production.

It is also worth to mention that impairment of the cannabinoid system may be important for the development of autoimmune diseases. Analysing CB_2R gene polymorphism, Sipe et al. (77) found that CB_2R 188-189 GG/GG homozygotes characterize by about twofold reduction of endocannabinoid-induced inhibition of T-cell proliferation compared with CB_2R 188-189 AA/AA homozygotes. It was also observed that patients with autoimmune diseases, including also subjects with systemic lupus erythematosus and rheumatoid arthritis had increased prevalence of the homozygous CB_2R GG/GG genotype (77).

Cannabinoid system as a possible target for future therapy in skin disease

Inflammatory skin diseases

As mentioned above, cannabinoids seem to have immunosuppressive properties and could be considered as potential anti-inflammatory drugs. Recently, Karsak et al. (87) reported that the endocannabinoid system could be involved in attenuation of allergic response to contact allergens. In their experiments, double knockout mouse, without expression of CB_1R and CB_2R ($CB_1R^{-/-}/CB_2R^{-/-}$), stimulated by 2,4-dinitrofluorobenzen, an obligate contact allergen, developed significantly more severe ear dermatitis

compared with wild-type mouse (87). Increased level of granulocytes and higher activity of myeloperoxidase, an indicative of enhanced neutrophil recruitment, were observed in knockout group compared with wild-type one. Moreover, knockout mouse demonstrated elevated number of MHC II antigen-positive cells in the inflamed area. Remarkably, 2,4-dinitrofluorobenzen treatment resulted in significant elevation of 2-AG and AEA levels in the skin. Interestingly, experiments with single deletion of either CB_1R or CB_2R revealed that both receptors are involved in the attenuation of contact allergic reaction (87). These results were confirmed by the use of CB_1R and CB_2R antagonists that induced increase in ear swelling in treated mouse compared with controls. Furthermore, a significantly decreased allergic response was observed in FAAH knockout mouse with retarded degradation of AEA (87). Finally, Karsak et al. (87) suggested that immunosuppressive effect of cannabinoid agonists in allergic inflammation may be related to monocyte chemotactic protein 2/chemokine (C-C motif) ligand 8 (MCP-2/CCL8), as *in vitro* experiments showed dynamic regulation of MCP-2/CCL8 production in activated keratinocytes through CBRs. Moreover, PEA has been demonstrated to down-modulate mast cell degranulation induced either by neurogenic (substance P) or immune-mediated stimuli, both *in vitro* and *in vivo* (88,89). Interestingly, it was also observed that substance P induced bronchoconstriction and airway oedema could be alleviated by CB_2R activation (90). In addition, activation of peripheral CB_2R decreased the spinal cord inflammation in animal model of multiple sclerosis (91,92). These observations carried out in different organs indirectly may support the idea that cannabinoids could also be important in the reduction of cutaneous inflammation.

However, the role of CB_2R in the cutaneous inflammation remains controversial. Oka et al. (93) reported activation of CB_2R during inflammation. Similarly to Karsak et al. (87), they found that the amount of 2-AG was markedly augmented in inflamed mouse ear, however, AEA level did not change markedly. Furthermore, treatment with a selective CB_2R antagonist blocked the ear swelling as well as reduced production of leukotriene B_4 and the infiltration of neutrophils in the mouse ear, whereas application of 2-AG to the mouse ear evoked swelling, the reaction that could be mediated by NO (93). In agreement with the study by Oka et al. (93), Ueda et al. (94) demonstrated that administration of JTE-907, an inverse CB_2R antagonist, and SR144528, a CB_2R antagonist, to DFNB treated mouse suppressed allergic inflammation. In another study, two selective CB_2R antagonists, AM1241 and JWH133, were shown to reduce the secretagogue compound 48/80-evoked ear oedema *in vivo* (95). It was suggested that 2-AG may induce migration of eosinophils and macrophages through CB_2R mechanism (94). Possibly, as supposed by Karsak et al. (87), CB_2R antagonism may be initially beneficial but detrimental upon chronic blockade.

Pruritus

Pruritus is considered as an unpleasant, localized or generalized sensation leading to intensive scratching or rubbing. Many patients consider itching as one of the most bothersome symptoms, sometimes even more unpleasant than pain. Pruritus is the most common symptom of different skin diseases, but may also accompany many systemic disorders. Although numerous antipruritic regimens exist, they frequently demonstrate limited efficacy and thus any new treatment option is warmly welcomed (96).

Recently published data suggested that cannabinoids, besides antinociceptive properties, may also exert antipruritic effect. Clinical and histological evaluation of PEA action in cats with eosinophilic granuloma demonstrated that after one month of treatment, 64% of all animals given PEA showed improvement of pruritus, erythema and alopecia, and 67% revealed improvement of extent and severity of the lesion (97). In addition, using an acute allergic mouse model, Schlosburg et al. (98) found that suppression of the neuronal FAAH reduces the scratching response through the inhibition of AEA degradation and activation of CB_1R.

Regarding humans, an open-labelled, non-controlled, prospective cohort study in a group of nearly 2500 subjects with atopic eczema demonstrated that a cream containing PEA significantly decreased objective and subjective symptoms of atopic eczema and was well tolerated (99). A complete resolution of pruritus was noted in 38.3% of individuals and significant improvement in further 41% of studied patients (99). Dvorak et al. (100) reported that CBR agonists significantly reduced histamine-evoked itch and vasodilatation by applying them topically before administration of histamine. In addition, co-administration of selective CB_1R agonists with histamine markedly reduced the axon reflex flare response (100,101). Antipruritic efficacy of cannabinoids is also supported by the results of the pilot study on patients with uremic pruritus (102,103). In an open label fashion, it was observed that twice daily application of a cream containing AEA and PEA for 3 weeks resulted in complete elimination of this symptom in 38.1% patients and significant reduction of its intensity in further 52.4% (102,103). In another open application study on 22 patients with prurigo, lichen simplex or refractory pruritus applying an emollient cream containing PEA, 63.6% of subjects reported marked relief of itching (104). The average reduction of itch was 86.4%. The therapy was well tolerated by all patients; neither burning nor contact dermatitis was observed (104).

Although well planned, double blinded, placebo-controlled studies on the efficacy of endocannabinoids in

the treatment of pruritus are still lacking, it seems that cannabinoids could be considered as potential therapeutic option for patients with pruritus who failed to other treatment modalities.

Thus, endocannabinoids seem to be promising agents for this symptom, although next, randomized, placebo-controlled studies are needed to confirm this advantageous effect.

Pain

Endocannabinoids are also important for modulation of pain perception. Activation of peripheral CB_1R attenuated dose-dependently existing hyperalgesia produced by a mild heat injury (105). In addition, selective activation of peripheral CB_2R produced antiallodynic activity in a rodent model of post-incisional pain (106,107). Simultaneous activation of peripheral CB_1R and CB_2R resulted in a synergistic inhibition of peripheral pain transmission (1). It also seems that endocannabinoids interact with PPARα agonists to reduce acute pain behaviours in a synergistic manner (108). Thus, cannabinoids might be considered as potential analgesic drugs. However, conversely, Costa et al. (109) demonstrated that also CB_1R antagonist may be of benefit when treating neuropathic pain, as this group showed that repeated oral administration of rimonabant (SR141716), a selective CB_1R antagonist, attenuated both thermal and mechanical hyperalgesia in rats with chronic constriction injury of the sciatic nerve. This effect could be explained by the myelin repair and subsequent long-lasting functional nerve recovery induced by rimonabant (109).

Interestingly, in another study, Costa et al. (15) reported that anti-hyperalgesical effect of PEA in mouse is mediated independently by three types of receptors: CB_1R, PPAR-γ and TRPV-1 and inhibition of one of these receptors only partially decreased the anti-hyperalgesic effect of PEA. Accordingly, only a combination of antagonists to all three receptors was able to completely reverse the anti-hyperalgesic property of PEA (15).

Some authors suggested that instead of direct activation of CB_1R by exogenous agonists, inhibition of FAAH is even more promising in pain treatment (110–113). It seems that disruption of FAAH function augments CB_1 signalling only in nervous system regions that are persistently stimulated, situation that is typically found in chronic pain. It is believed that inhibition of FAAH would result in analgesia without side effects accompanying typically activation of CB_1R (110,114). In addition, it was shown that inhibition of monoacylglycerol lipase, an another enzyme responsible for degradation of endocannabinoids, may also produce analgesia (65).

Cutaneous malignancies

Already in 1970s, exogenous cannabinoids were considered as potential anticancer drugs (115). Up to date there is an increasing knowledge about the anti-tumor effect of endocannabinoids, that may induce apoptosis, inhibit tumor cell proliferation and migration, diminish the expression of proangiogenic agents and their receptors, reduce vascular hyperplasia and modulate signal transduction in different cell lines (67,116). These effects were observed in gliomas, lymphomas, prostate, breast, lung and pancreatic cancers as well as in skin malignancies (7,117). The role of endocannabinoids in cancer therapy concentrates mainly on proapoptotic properties for cancer cells. There are many evidences that endocannabinoids may remodulate signal transduction in different tumors, and this could lead to increased synthesis of sphingolipids, ceramides, p8 protein and downstream of stress related genes (ATF-4, CHOP and TRB3), activation of Raf-1/MAP kinase and inhibition of Akt, c-Jun NH_2 terminal kinase and p38 MAP kinase. Inhibitory effect on tumor cells is most probably caused by inhibition of adenyl cyclase and the cAMP/PKA pathway, induction of the cyclin dependent kinase inhibitor $p27^{kip1}$, decrease in epidermal growth factor receptor (EGF-R) expression or its kinase activity and decrease in activity and/or expression of nerve growth factor or vascular endothelial growth factor receptor 2 (7,116,117).

Melanoma still remains a management challenge. Many patients, especially with deeply infiltrating tumors, demonstrate poor prognosis despite the aggressive, anticancer treatment. Application of Δ^9-THC and its analogue, nabilone, have been proposed by several authors (116,118–120) as additional therapy to prevent chemotherapy-induced nausea and vomiting, appetite stimulation and pain inhibition (115,121). Interestingly, recent observations also suggested that cannabinoids may be potent anti-tumor drugs.

Blazquez et al. (116) observed that melanoma cells of mouse and human origin expressed CB_1R and CB_2R. Furthermore, *in vitro* experiments on A353 and MelJuso melanoma cell lines demonstrated that cannabinoids significantly decreased the number of viable melanoma cells in cultures by inducing apoptosis, and selective antagonists for CB_1R (SR141716) and CB_2R (SR144528, AM630) prevented this effect. Interestingly, proliferation of normal melanocyte cell lines was not inhibited, although they also expressed CB_1R (116). In addition, it was clearly documented that CB_2R agonists inhibit melanoma progression and metastatic spreading in mouse (116).

Endocannabinoids may also be beneficial in non-melanoma skin cancers. CB_1R and CB_2R were shown to be expressed in benign (papillomas) and malignant skin tumor cells (squamous cell carcinoma) in mouse and humans (67). Remarkably, activation of CBRs in cell culture experiments induced apoptosis in tumorigenic epidermal cells, whereas the viability of normal epidermal cells remained unaffected (67). Furthermore, treatment with CB_1R/CB_2R

(WIN-55,212-2) or selective CB_2R (JWH-133) agonists resulted in significant growth inhibition of malignant tumors (67). Cannabinoid-treated tumors showed an increased number of apoptotic cells and impaired vascularization (pattern of blood vessels characterized predominantly by narrow capillaries) as well as decreased expression of proangiogenic factors (vascular endothelial growth factor, placental growth factor and angiopoietin 2) (67). In addition, cannabinoid-treated tumors demonstrated abrogation of EGF-R function (67), an important component in the development of non-melanoma skin cancers triggering the angiogenic switch necessary for skin tumor growth (121,122).

On the other hand, the study by Zheng et al. (123) suggested that cannabinoids may also be involved in the early stages of malignant transformation. These authors observed that both CBRs, CB_1R and CB_2R, are activated by UVA and UVB, resulting in NF-κB activation and elevated level of TNF-α (120). These results might be connected with a rapid phosphorylation and internalization of both CBRs induced by UVB irradiation. It was also shown that the skin from $CB_1^{-/-}/CB_2^{-/-}$ knockout mouse is resistant to UVB-evoked inflammation (123). Importantly, $CB_1R^{-/-}/CB_2R^{-/-}$ mouse was also more resistant to UVB-induced papilloma development (123). Furthermore, papillomas found in wild-type mouse were more numerous and larger compared with those in $CB_1R^{-/-}/CB_2R^{-/-}$ mouse (123).

In another study (122), low doses of Δ^9-THC were shown to improve the efficiency of Kaposi's sarcoma-associated herpes virus (KSHV, also named human herpes virus 8) to infect human dermal microvascular endothelial cells (HMVEC) *in vitro*, suggesting that cannabinoid system could be involved in spreading of some oncogenic viruses. This observation could be linked with the immunoinhibitory effect of endocannabinoids. It was observed that Δ^9-THC induced KSHV replication in endothelial cells through up-regulation of *ORF50* expression, the major switch gene for KSHV from latency to the lytic cycle (122). Finally, Δ^9-THC enhanced the adhesion between B lymphocytes and HMVEC by increasing the expression of PECAM-1. These findings may indicate that Δ^9-THC can promote viral transmission (122).

Conclusions

On the basis of the current knowledge, therapeutic possibilities of cannabinoid usage in skin diseases seem to be unquestionable. Possibly, in the future, cannabinoids will be widely applied to treat pruritus, inflammatory skin diseases and even skin cancers. However, our understanding of the role of cannabinoid system in the skin is still not completed, and next studies evaluating this exciting aspect of cutaneous biology are highly required.

Conflict of interests

None to disclosure.

Founding sources

None.

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