Role of Lipid Rafts/Caveolae in the Anticancer Effect of Endocannabinoids

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Abstract: The endocannabinoid system comprises the cannabinoid receptors type 1 (CB1) and type 2 (CB2), their endogenous ligands (endocannabinoids) and the whole apparatus appointed of their synthesis and degradation. Recent studies investigated the possibility that drugs targeting the endocannabinoid system might be used to retard or block cancer growth. CB1, CB2 and metabolic enzymes of endocannabinoids, function in the context of lipid rafts, specialized membrane microdomains enriched in cholesterol, sphingolipids and glycosphingolipids which may be important in modulating signal transduction. Here, we analysed the role of lipid rafts/caveolae in the intracellular signaling and trafficking of cannabinoid receptor agonist in cancer cells. Perturbation of lipid rafts/caveolae may in fact represent a useful tool for the development of a novel therapy for endocannabinoids-related diseases, such as cancer. Also, we report the more recent developments of endocannabinoids in cancer drug discovery.

Keywords: Endocannabinoids, cancer, lipid rafts.

1. INTRODUCTION

The recreational use of Cannabis Sativa preparations is known to most people [1]. However, the medicinal use of Cannabis also has a millenarian history that has been re-examined only very recently [2]. This long history of Cannabis medical use has resulted in the development of pharmaceutical drugs, such as Cesamet, Marinol and Sativex. The first two preparations are approved by FDA and can be prescribed to reduce chemotherapy-induced nausea and vomiting. Dronabinol is also approved for the treatment of anorexia and weight loss associated with AIDS. These drugs are based on Δ^9-tetrahydrocannabinol (THC, Fig. **1**), which in 1964 was identified by Mechoulam and coworkers as the major psychoactive component of cannabis. The third medicinal cannabis product, Sativex (a combination of THC and cannabidiol) is prescribed for the symptomatic relief of neuropathic pain in multiple sclerosis and as an analgesic treatment for patients with advanced cancer [3].

To date, some 60 plant terpenophenols more or less related to THC have been isolated and defined cannabinoids Δ^9-tetrahydrocannabinol, the primary psychoactive ingredient of cannabis, for its potency and abundance, is the most important [4].

2. THE ENDOCANNABINOID SYSTEM

2.1. Cannabinoid Receptors

Thus far, two cannabinoid-specific receptors have been cloned and characterized from mammalian tissues, the seven transmembrane G protein-coupled cannabinoid receptors type 1 (CB1 receptor), [5] and type 2 (CB2 receptor) [6]. Whereas the CB1 receptor expression is abundant in the central nervous system, the CB2 receptor is almost exclusively expressed in the immune system. The CB1 receptor is also expressed in peripheral nerve terminals and various extraneuronal sites such as the testis, uterus, eye, vascular endothelial, spleen and adipocytes [7-10].

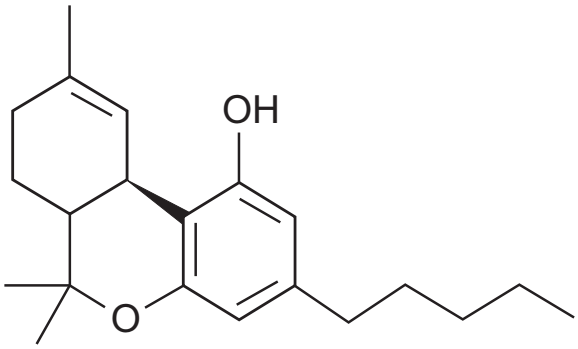

Fig. (1). Chemical structure of Δ^9-tetrahydrocannabinol.

CB1 and CB2 receptors share only 44% overall identity and 68% within the transmembrane domains. Both cannabinoid receptors are coupled to G proteins, mostly of the $G_{i/o}$ type, through whose α subunit they inhibit the activity of adenylate cyclases and stimulate mitogen-activated protein kinases. However, additional studies established that cannabinoid receptors were also coupled to ion channels, resultant in the inhibition of Ca2+ influx through N type calcium channels [11]. CB1 receptors are also implicated in the activation of both phospholipase C (*via* the βγ subunits of the G protein) and PI-3-kinase. CB2 receptors, on the other hand, trigger a sustained activation of ceramide biosynthesis [12].

More recently, a type-3 (CB3 receptor or GPR55) that shows high levels of expression in human striatum, was identified as a putative cannabinoid receptor [13].

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A genetics study on the origins of the cannabinoid system has concluded that there is no significant sequence similarity between GPR55 and CB1 or CB2. In fact, GPR55 displays low sequence identity in both CB1 (13.5%) and CB2 (14.4%) receptor [14].

Because of the large body of conflicting pharmacological data, no conclusive decision can yet be reached about whether GPR55 should be classified as a novel cannabinoid receptor [15]. However, further studies elucidating whether the GPR55 receptor is a true cannabinoid receptor should be conducted 2.2.

2.2. Endocannabinoids

Several endogenous fatty-acid ligands, known as endocannabinoids, have been shown to bind and activate cannabinoid receptors. The first to be discovered, in 1992, was arachidonoylethanolamide (anandamide, AEA) followed by 2-arachidonoylglycerol (2-AG). Both these compounds are derivates of arachidonic acid conjugated with ethanolamine or glycerol and are able to bind to CB1 and CB2 receptors, although with differences in affinities and activation efficacies [8].

AEA, but not 2-AG, is also an agonist of the transient receptor potential vanilloid 1 (TRPV1), which is the natural target of capsaicin, the natural product responsible for the pungency of hot chilli peppers [16].

Additional targets of eCBs are the peroxisome proliferator-activated receptors (PPARs). PPARs are ligand-activated transcription factors that constitute part of the nuclear receptor family. To date, three different PPAR subtypes (α, γ, and β/δ), have been cloned [17]. AEA and 2-AG have each already been reported to activate two PPAR receptors: PPARα and PPARγ (AEA) and PPARγ and PPARβ/δ (2-AG). Overall, the potencies of endocannabinoids and their metabolites as PPAR agonists or antagonists are relatively low compared with their potencies as agonists of canonical cannabinoid CB1/CB2 receptors. This might be taken as evidence that endocannabinoids are poor candidates as PPAR ligands *in vivo* [15].

During the last few years, several other bioactive lipid mediators have been described; they appear to be active, through CB1 and/or CB2 receptors and confer specific pharmacological effects *in vivo*.

Specifically, the compounds are 2-arachidonoyl-glyceryl-ether (noladin ether), o-arachidonoyl-ethanolamine (virodhamine), N- arachidonoyl-dopamine, and oleamide [10, 18-20] (Fig. **2**).

Endocannabinoid are very lipophilic and thus cannot be stored in vescicles like other neurotransmitters but are produced on demand from phospholipids precursors of cell membrane. After the synthesis and immediate release, endocannabinoids can activate cannabinoid receptors, seem to act on molecular targets in an autocrine or paracrine manner and are subsequently inactivated by cellular re-upatke [21].

Consequently, the regulation of endocannabinoid signaling is tightly controlled by their synthesis, release, uptake and degradation [22]. Several different stimuli, including membrane depolarization and increased intracellular Ca^{2+} and/or receptor stimulation, can activate complex enzymatic machineries, which lead to the cleavage of membrane phospholipids and eventually to the synthesis of endocannabinoids [23].

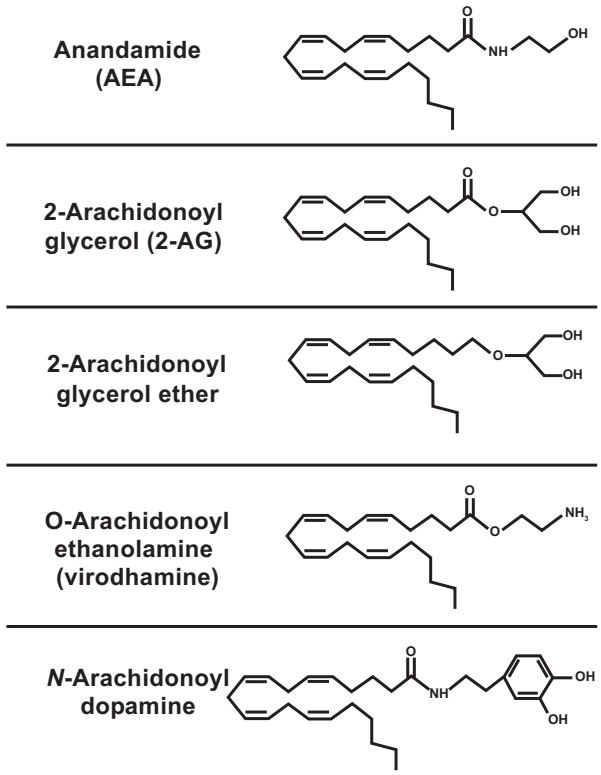

Fig. (2). Chemical structure of endogenous cannabinoid.

After synthesis, endocannabinoids can activate cannabinoid receptors and release into the extracellular space or directly within the cell membrane. Endocannabinoid signaling is limited by very efficient degradation processes, involving facilitated uptake from the extracellular space into the cell and enzymatic catabolism mediated by specific intracellular enzymes. The molecular nature of the carrier protein(s) involved in endocannabinoid uptake has not yet been elucidated. However, the enzymes able to degrade endocannabinoids are well characterized. They are fatty acid amide hydrolase (FAAH) for anandamide and related compounds [24] and monoglycerol lipase for 2-AG [25], although other enzymes might be partially involved in the degradation of this last compound [26].

An interesting aspect of endocannabinoid activity is the rapid induction of their synthesis, receptor activation, and degradation [27]. The endocannabinoid system has thus been suggested to act on demand, and exerts its modulatory actions only when and where it is needed.

2.3. Central and Peripheral Functions of Endocannabinoid System

Cannabinoid receptors, endocannabinoids and the whole apparatus appointed of their synthesis and degradation represent the elements of a novel endogenous signaling system (the endocannabinoid system) which is implicated in an overabundance of physiological functions [22, 28]. During the last few years, a notable quantity of data has been reported to understand the biological roles of this system in more detail.

In general, endocannabinoid system serves several functions under physiological conditions. In the CNS, endocannabinoids intervene in the regulation of cognitive functions and emotions in neuronal circuits of the cortex, hippocampus and amygdale and to the reinforcement of substances of abuse in the mesolimbic system [29].

Endocannabinoids also modulate the control of movement and posture [30], the regulation of pain perception [31] and cardiovascular [32], gastrointestinal [33], respiratory and reproductive functions [34]. CB2 receptors, instead, are involved in cellular and particularly humoral immune response, with possible implications for (neuro)inflammation and chronic pain [35].

Apart from the possible physiological functions of the endocannabinoid system briefly described above, endocannabinoid signaling undergoes dramatic tissue and blood changes under pathological conditions. Higher endocannabinoid levels are found in the case of experimental models of neurodegenerative disease, like Parkinson's and Alzheimer's disease and amyotropic lateral sclerosis, in gastrointestinal disorders like colon inflammation and in eating and metabolic disorders like anorexia nervosa, binge-eating disorders and obesity [36]. Finally, yet importantly, elevated levels of endocannabinoids have been observed in several types of cancer like glioblastoma [37], meningioma [37], colon [38] and prostate [39] carcinoma, colon polyps [38] and pituitary adenoma [40] as compared to their normal counterparts, suggesting a function of the endocannabinoid as potential tumor growth inhibitors.

3. LIPID RAFTS AND CAVEOLAE

Lipid rafts are receiving increasing attention as devices that regulate membrane function in eukaryotic cells and have changed our view of membrane organization. Lipid rafts are dynamic assemblies of proteins and lipids that float freely within the liquid-disordered bilayer of cellular membranes but can also cluster to form larger, ordered platforms. Lipid rafts are planar domains in the plasma membrane that are rich in sphingolipids, cholesterol, plasmenylethanolamine and arachidonic acid. They are defined by the insolubility of their components in cold non-ionic detergents (like Triton X-100) [41, 42].

Thus, the presence of liquid-ordered microdomains in cells transforms the classical membrane fluid mosaic model of Singer and Nicholson into a more complex system, where proteins and lipid rafts diffuse laterally within a two-dimensional liquid. The raft concept has long been controversial, largely because it has been difficult to prove definitively that rafts exist in living cells. But recent studies with improved methodology have dispelled most of these doubts [43]. One of the most important properties of lipid rafts is that they can include or exclude proteins to variable extents. Proteins with raft affinity include glycosylphosphatidylinositol (GPI)-anchored proteins [44, 45], doubly acylated proteins, such as Src-family kinases or the α-subunits of heterotrimeric G proteins [46], cholesterol-linked and transmembrane proteins, particularly palmitoylated ones [44].

The distribution of lipid rafts over the cell surface depends on the cell type. In polarized epithelial cells and neurons, lipid rafts accumulate in the apical and axonal plasma membrane, respectively. Basolateral and somatodendritic membranes also contain rafts, but in smaller amounts [41]. In lymphocytes and fibroblasts, rafts are distributed over the cell surface without an obvious polarity. Raft lipids are most abundant at the plasma membrane, but can also be found in the biosynthetic and endocytic pathways. Whereas cholesterol is synthesized in the endoplasmic reticulum (ER), sphingolipid synthesis and head-group modification are completed largely in the Golgi [47]. As these data predict, cholesterol–sphingolipid rafts first assemble in the Golgi. Movement of lipid rafts out of the Golgi seems to be mainly towards the plasma membrane, as vesicles going back to the ER contain little sphingomyelin and cholesterol [48]. The inclusion of proteins into rafts is important for polarized delivery to the cell surface in many cell types [41, 49, 50]. Lipid raft trafficking does not end with surface delivery rafts being continuously endocytosed from the plasma membrane [51]. From early endosomes, rafts either recycle directly back to the cell surface or return indirectly through recycling endosomes, which could also deliver rafts to the Golgi [52].

The most important role of rafts at the cell surface may be their function in signal transduction. It is well established that, in the case of tyrosine kinase signaling, adaptors, scaffolds and enzymes are recruited to the cytoplasmic side of the plasma membrane as a result of ligand activation [53]. One way to consider rafts is that they form concentrating platforms for individual receptors, activated by ligand binding. If receptor activation takes place in a lipid raft, the signaling complex is protected from non-raft enzymes such as membrane phosphatases that otherwise could affect the signaling process. In general, raft binding recruits proteins to a new micro-environment, where the phosphorylation state can be modified by local kinases and phosphatases, resulting in downstream signalling. To highlight these principles, examples of signaling pathways that involve lipid rafts are Immunoglobulin E signalling, T-cell antigen receptor signaling and Ras signaling [43].

One subset of lipid rafts is found in cell surface invagination called caveolae. These flask-shaped plasma membrane invaginations were first identified in 1950s on the basis of their morphology. Caveolae are formed from lipid rafts by polymerization of caveolins, a family of integral membrane proteins that tightly bind cholesterol and that are necessary for the formation of these organelles [41, 43]. The general function of caveolae is not clear; they have been shown to play an important role in the regulation of various

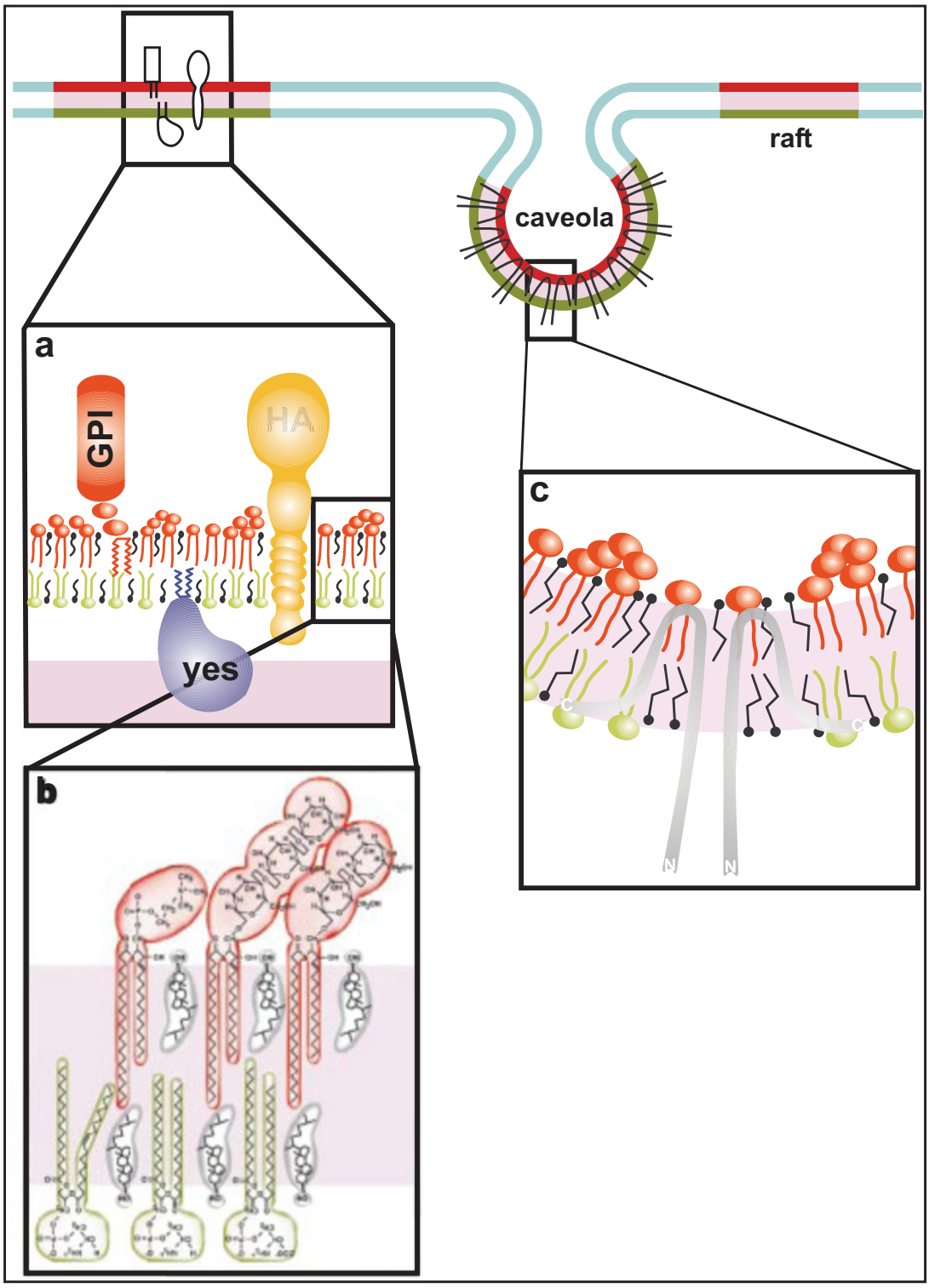

Fig. (3). Model for the organization of rafts and caveolae in the plasma membrane. The rafts (red) segregate from the other regions (blue) of the bilayer. **a**) Rafts contain proteins attached to the exoplasmic leaflet of the bilayer by their GPI anchors, proteins binding to the cytoplasmic leaflet by acyl tails or proteins associating through their transmembrane domains. **b**) Caveolae are formed by self associating caveolin molecules making a hairpin loop in the membrane. Interactions with raft lipids may be mediated by binding to cholesterol and by acylation of C-terminal cysteines. **c**) The lipid bilayer in rafts is asymmetric, with sphingomyelin (red) and glycosphingolipids (red) enriched in the exoplasmic leaflet and glycerolipids in the cytoplasmic leaflet. Cholesterol (grey) is present in both leaflets and fills the space under the head groups of sphingolipids or extends the interdigitating fatty acyl chain in the apposing leaflet. From: Simons K, Ikonen E Functional rafts in cell membranes. Nature 1997; 387: 569-572 [41].

cellular functions including organization of cell signaling machinery such as receptor tyrosine kinases and GPCRs, cholesterol transport, potocytosis, endocytosis cell polarization and migration [54, 55].

Interestingly, down-regulation of cav1 protein expression leads to deregulation of signaling and this event seems to play a critical role during tumorigenesis [56-58]. Recently, a role for cav1 has emerged as a modulator of signaling [56]. Cav1 interacts and modulates G protein α subunits, H-Ras, Src-family tyrosine kinase, PKC isoforms, EGF-R, Neu [56], PKA catalytic subunits [59] and insulin receptor [60]. However, the potential role of lipid rafts in the progression of solid tumors is poorly understood but the role of caveolin-1 in signaling mechanisms relevant to cancer has been extensively studied. Caveolin 1 has been identified as a marker of aggressive disease in prostate, pancreatic, and esophageal carcinoma [61]. In model systems, caveolin 1 was demonstrated to promote progression to the metastatic phenotype [62]. Interestingly, by using animal model approach it has been shown that caveolin 1 is a potent suppressor of mammary tumor growth and metastasis and it has been shown to regulate breast tumor growth and metastasis of breast tumor [63]. However the exact functional role of caveolin 1 remains controversial.

4. LIPID RAFTS AND ENDOCANNABINOID SYSTEM

Lipid rafts and caveolae are available as important platforms for the regulation of a variety of processes linked to the endocannabinoid system [64].

Recent evidence suggests that lipid rafts/caveolae play a role in the uptake and recycling of anandamide [65]. The pharmacologic disruption of the lipid raft microorganization results in an attenuation of AEA uptake [66, 67]. This and other studies suggest a new model for AEA uptake that implicates lipid raft/caveolae organization. The enzyme primarily responsible for anandamide metabolism, FAAH, is excluded from lipid rafts. However, the AEA metabolites arachidonic acid (AA) and ethanolamine become enriched in the lipid raft portion of the cell. These metabolites, AA and ethanolamine, can then be utilized by the cell for recycling, synthesis, and release of new AEA molecules [68]. There is some preliminary evidence that makes it reasonable to propose that anandamide metabolites enriched in lipid rafts may act as precursors to anandamide synthesis.

The disruption of lipid rafts results in the attenuation of AEA and 2-AG formation. These results imply that AEA and 2-AG formation and release may take place in the lipid raft/caveolae microdomains of the cell membrane [69].

The finding that AEA, 2-AG and the synthetic machinery for their synthesis and binding are localized to the lipid raft portion of the cell suggests that the production of 2-AG is localized within lipid rafts suggesting that 2-AG synthesis *via* diacylglycerol occurs within these microdomains [70].

Lipid rafts/caveolae have also been implicated in the modulation of the CB1 receptor binding and signaling [71], but the topology of CB1R and its proximal signaling partners in respect to lipid rafts has not been addressed [72]. Sarnataro *et al.* showed that CB1 receptor is associated with lipid rafts. Cholesterol depletion by methyl-β-cyclodextrin treatment (which extracts cholesterol from the plasma membrane) strongly reduces the flotation of the protein on the raft-fractions of sucrose density gradients suggesting that CB1 raft-association is cholesterol dependent [73]. However, it has been shown that CB1 receptor and endocannabinoid transporters are probably localized within lipid rafts, at variance with CB2 receptor. In immune and neuronal cells, lipid rafts control CB1, but not CB2 and endocannabinoid transport [74]. Intriguingly, Bari *et al.* [75] found that Type-1 cannabinoid receptors colocalize with caveolin-1 in neuronal cells, suggesting a strong link between CB1 receptor and cav1, that seems interesting because caveolae play a role in neurodegenerative diseases, [76] like endocannabinoids, that have been shown to interfere with these processes through CB1R-dependent mechanisms [77].

5. INTRACELLULAR TRAFFICKING AND SIGNALING OF CANNABINOID RECEPTORS IN CANCER CELLS: THE ROLE OF LIPID RAFTS

In the early 1970's, before the discovery of cannabinoid receptors and endocannabinoids, the anti-neoplastic activity of THC and its analogs was observed [78]. However, it was not until the last 15 years that the therapeutic potential of plant and synthetic and endogenous cannabinoids on various types of cancer cell was revisited. Initially considered to exert their anti-tumoral actions by proliferation arrest or apoptosis, cannabinoids and endocannabinoids are now emerging as suppressors of angiogenesis and tumor metastatic spreading [79-81]. Therefore, understanding the mechanism of intracellular trafficking of cannabinoid receptors, in the presence or absence of its agonists/antagonists will be important for the identification of novel molecular targets for cancer therapy. Several evidences suggest that lipid rafts might modulate the endocannabinoid signaling and CB1 receptor trafficking and function [65, 68, 74-78].

It has been shown that methyl-β-cyclodextrin, which extracts cholesterol from the plasma membrane, completely blocks anandamide-induced cell death in a variety of cells, including PC12, C6, HEK and HL-60 cells [82] and that anandamide induces apoptosis, at least in hepatoma cell line (Hep G2), interacting with cholesterol present in the cell membrane [83]. The involvement of raft-dependent pathway in ceramide formation, p38 and p42/44 MAPK activation and subsequent COX-2 expression by endocannabinoid analog in human neuroglioma cells has also been examined [84]. Moreover, to elucidate the mechanism of the antiproliferative CB1-mediated effects, the selective antagonist SR141716 were used in MDA-MB-231 human breast cancer cells. The authors report that cholesterol depletion by methyl-β-cyclodextrin strongly prevented SR141716-mediated antiproliferative effect, suggesting that SR141716 inhibits human breast cancer cell growth *via* a CB1 receptor lipid raft/caveolae-mediated mechanism [85]. deMorrow *et al.* demonstrated that the opposing actions of AEA and 2-AG on cholangiocarcinoma are cannabinoid receptor-independent, but lipid raft mediated pathway. Further, the authors have shown that the anti-proliferative/ pro-apoptotic actions of AEA are mediated by recruitment of the Fas death receptor into the lipid rafts [86].

Recently, Hamtiaux *et al.* confirmed in a neuroblastoma model the antiproliferative effects of AEA. This effect is independent of the known molecular *N*-acylethanolamine targets - cannabinoid, TRPV1, PPARα/γ or GPR55 receptors – activation, but relies on a lipid raft-mediated mechanism. Hence, these data open the way to potential *N*-acylethanolamine-based treatments aiming at reducing cancer cell proliferation through inhibition of their degradation [87]. Human melanoma cells preincubated with the lipid raft disruptor methylcyclodestrin and treated with WIN were rescued from death. WIN induced the activation of caspases and phosphorylation of ERK that were attenuated in cultures treated with methylcyclodextrin. Membrane lipid raft complex-mediated antimitogenic effect of WIN in melanoma could represent a potential target for the melanoma treatment [88].

CONCLUSIONS

The endocannabinoid system was discovered through research into Δ9-tetrahydrocannabinol (9-THC), the active ingredient in cannabis. In the present review, numerous studies have suggested that cannabinoids might directly inhibit cancer growth. The proposed mechanisms are complex and may involve induction of apoptosis in tumor cells, anti-proliferative action, and an anti-metastatic effect through inhibition of angiogenesis and tumor cell migration.

The development of several pharmaceutical formulations as well as new cannabinoids is an important step in improving the quality of treatment and quality of life of the cancer patient.

Currently employed therapies for the tumors (surgery, radiotherapy, chemotherapy, immunotherapy, gene therapy) are generally ineffective or at best palliative. Therefore, today the medical oncology has different formulations as well as new cannabinoids with the same efficacy and the tolerability profile and can then choose the best option for each patient in different clinical situations.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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