Involvement of Cannabinoids in Cellular Proliferation

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Abstract: The endogenous canabinoid system (ECS) is involved in the regulation of an important number of central and peripheral physiological effects. Among all these functions, the control of the cellular proliferation has become a focus of major attention as opening new therapeutic possibilities for the use of cannabinoids as potential antitumor agents. The capacity of endogenous and synthetic cannabinoids to induce apoptosis of different tumoral cells in culture and *in vivo*, the mechanism underlying and the potential therapeutic applications are discussed in this review.

Keywords: Endogenous cannabinoid system, cellular proliferation, antitumoral properties of cannabinoids, tumoral cells, cancer.

1. INTRODUCTION

In the recent years much progress has been done about the potential use of *Cannabis sativa*-derived compounds as well as cannabimimetic fatty acid derivatives as therapeutic agents. Cannabinoid agonists may regulate many physiological functions and thus they might be useful in the treatment of pathological conditions associated with such functions. The use of either endogenous, synthetic or naturally occurring cannabinoids in some clinical disorders as bronchial asthma, epilepsy, glaucoma or motor disorders, has begun to emerge [1, 2]. Recently, a role for cannabinoids in the regulation of cellular proliferation has been proposed [3] and these findings open new therapeutic possibilities for the use of cannabinoids as potential antitumor agents. Although the antineoplasic activity of (-)-Δ^9-tetrahydrocannabinol (THC, (Fig. **1**)) in adenocarcinomas and leukaemic cells was observed more than twenty years ago [4-7 and reviewed in 8], it is not until the last few years that the role of cannabinoids in the regulation of cellular growth is being extensively studied. The capacity of endogenous and synthetic cannabinoids to regulate proliferation in different cells, the ability of cannabinoid agonists to reduce tumor growth *in vivo* as well as the main structure-affinity relationship studies carried out in the different types of cannabimimetics with antitumoral properties are discussed in this review. Some clinical studies are also presented.

2. CANNABIMIMETIC COMPOUNDS WITH ANTIPROLIFERATIVE PROPERTIES

Within the current renewed interest in cannabinoids for medicinal purposes, their beneficial effects in oncology constitutes one of the main issues endowed with important potential therapeutic applications. So far, cannabimimetic compounds have demonstrated remarkable capacities for the management of tumoral processes in different experimental models. These effects include not only the reduction of tumoral cells due to their direct antiproliferative effects but also they could represent an important new strategy for the treatment of the wide variety of cancer-related disorders including analgesia, mood elevation, muscle relaxation, relief of insomnia and alleviation of the chemotherapy induced nausea. Since these aspects are clearly beyond the scope of this review and have been recently addressed [9], we will focus on the direct antiproliferative properties exerted by cannabinoids.

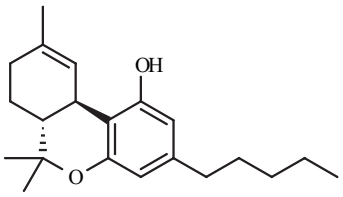

Fig. (1). Structure of (-)-Δ^9-tetrahydrocannabinol (THC).

The first reported result related to this issue was the antineoplasic activity of THC, the active principle in *Cannabis sativa*, in the early seventies [4-6]. This result, although interesting, stayed without major development almost until the last five years, probably hampered by the psychotropic side effects that the treatment with THC could induce. However, at the present moment and based on the more solid bases provided by the last advances on the knowledge of the endogenous cannabinoid system (ECS), the attractive possibility of the ECS as a new putative target for the management of tumoral diseases has become a focus of intense and growing research.

In fact, the current knowledge about the ECS has allowed to postulate diverse hypotheses aimed at taking advance of the exogenous regulation of ECS as a strategy for the treatment of cancer but avoiding the undesirable

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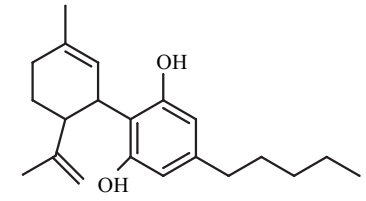
psychotropic effects that some cannabinoid agonists could have, aspect which reflects the main drawback attributed to the use of THC. Two hypothesis are being currently taken into consideration as potential ways to activate the ECS without inducing any undesirable side effects: (i) selective activation of CB_2 receptor and (ii) increase of the endogenous cannabinoid tone. Both objectives can be fulfilled by the use of either naturally-occurring or synthetic cannabinoids.

2.1. Naturally Occurring Cannabinoids

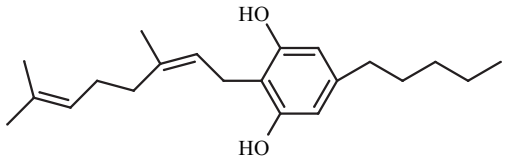
This class of compounds includes both the vegetal and the animal origin cannabinoids. Vegetal cannabinoids include the more than sixty cannabinoids present in *C. sativa*, all of them structurally closely related to THC. Many of these compounds differ only in a single functional group or the number or position of insaturations and they are likely to be midpoints along the cannabinoid metabolism such as degradation products, precursors or byproducts [10]. Cannabinoids of animal origin display, however, a remarkable different structure, belonging to the group of eicosanoids. They can be classified as a class of lipid mediators derived from arachidonic acid.

2.1.1. Cannabinoids of Vegetal Origin

Although the most representative compound is the widely studied THC, other compounds with interesting antitumor properties and devoid of side effects have also been described. THC has shown an important ability to inhibit the growth of different tumoral cells. Besides the early studies that indicated the anti-neoplasic capacity of THC towards leukaemia cells [11, 12], THC has also demonstrated to induce apoptosis in rat glioma C6 cells inoculated either intracerebrally or subcutaneously in rats or mice respectively, without any remarkable toxic effect [13] as well as in prostate cancer cells [14].



Cannabidiol



Cannabigerol

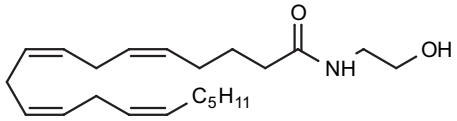
Fig. (2). Structures of cannabidiol and cannabigerol.

Among the other compounds closely related to THC, deserve special attention the non-psychotropic cannabinoids cannabidiol and cannabigerol (Fig. **2**). Cannabidiol was found to be less potent that its counter part THC in the same series of studies [5, 15 and reviewed in 12] whereas cannabigerol has showed its capacity to inhibit the proliferation of human oral epitheloid cells as well as the NIH3T3 fibroblasts [12, 16].

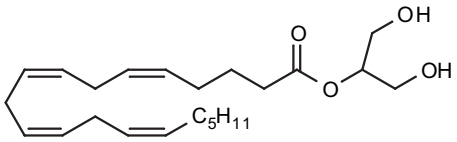
Regarding their mechanisms of action, it has been reported that their antitumor effects seem to be mediated by other mechanisms different from targeting the ECS [12], although this is not a surprising fact since also some of the antiproliferative effects of THC have been described as independent of the activation of the cannabinoid receptors or, at least, not reverted in presence of the cannabinoid antagonists developed so far [14, 17].

2.1.2. Cannabinoids of Animal Origin

The identification in the last decade of the main endocannabinoids in mammals (Fig. **3**), named anandamide (AEA, *N*-arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG), together with the previously known antitumoral effects of THC, led very soon to the hypothesis whether these compounds could also show antitumoral properties.



Anandamide (AEA)



2-Arachidonoylglycerol (2-AG)

Fig. (3). Structure of representative endocannabinoids.

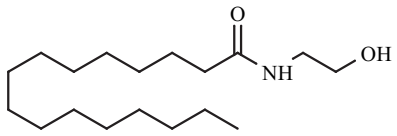
In this context, AEA has demonstrated a potent dose-dependent capacity to inhibit the proliferation of human breast cancer cells (HBCCs) [18] and also similar effects were described for 2-AG [12], being in this case the antimitogenic responses mainly mediated by the CB_1 cannabinoid receptor [12, 18, 19]. Similar results were obtained when studying the prostate cancer cell line DU145 [19].

In particular, AEA has been shown to inhibit the growth of the HBCCs MCF-7, T-47D and EFM-19 with IC_{50} values of 1.4, 1.9 and 2.1 μM, respectively [20, 21]. 2-AG has shown a similar capacity to inhibit the proliferation in MCF-7 cells (IC_{50} = 1.4 μM) and a slightly minor capacity in T-47D cells (IC_{50} = 5 μM). However, both compounds were able to induce a potent inhibition in DU-145 cells, with IC_{50} values in the nanomolar range (IC_{50} values between 100 and 300 nM) [19].

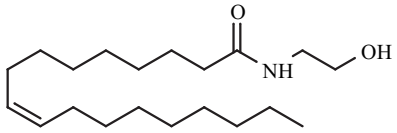
Among the AEA related compounds, the *N*-acylethanolamines oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) have been also a focus of in-depth research due to their ability to enhance the antiproliferative effects of AEA without inducing appreciable effects by themselves.

Regarding to this, OEA, present at high concentrations in EFM-19 cells, has shown a CB_1 dependent capacity, albeit weaker than AEA, as inhibitor of tumoral proliferation

with an IC_{50} value of 11.3 μM together with the ability to prevent the AEA degradation [21]. Additionally, a low ineffective concentration of OEA is able to produce a significant potentiation in the antiproliferative effects of AEA, response which was blocked in presence of SR141716A, showing that OEA might inhibit cell proliferation by raising the levels of AEA due to its action as a substrate competitor for the fatty acid amide hydrolase (FAAH) [21]. All these data, taken together, have allowed to propose AEA and OEA as autacoid suppressors of human cancer cell proliferation [21].



Palmithoylethanolamide (PEA)



Oleoylethanolamide (OEA)

Fig. (4). Structures of palmithoylethanolamide and oleoyl-ethanolamide.

PEA, which is also able to enhance the antiproliferative effects of AEA in HBCCs, seems to act as an entourage compound by inhibiting endogenous AEA degradation by competition for FAAH, similarly to OEA. Additionally, a second mechanism consisting of the downregulation of the expression of this enzyme has also been reported [22].

However, PEA, besides its effects upon FAAH, is also able to enhance those effects of AEA involving the vanilloid receptor (VR_1). Recently it has been reported that the interaction of PEA with VR_1, probably via an allosteric mechanism, could enhance the affinity of AEA for this receptor and thereby increasing its VR_1 mediated actions [23]. In fact, compounds able to activate both CB_1 and VR_1 receptors, such as arvanil (Fig. **5**), which is a vanilloid-cannabinoid hydrid (for recent reviews about VR_1 and the interaction between vanilloid and cannabinoid systems see [24, 25]), show higher antiproliferative activities than pure agonists of either receptor class. In keeping with this, PEA has demonstrated its ability to induce quite significant effects to increase the antiproliferative responses of the most representative VR_1 agonists (Fig. **6**) including olvanil, capsaicin or resiniferatoxin [26]. This enhancement of the functional effects exerted by these vanilloid agonists could be related with PEA acting as an allosteric modulator of VR_1 receptors [26].

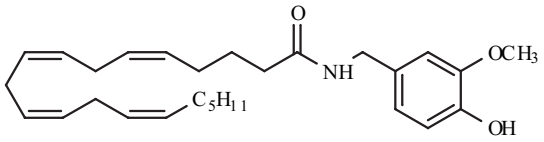


Fig. (5). Structure of arvanil.

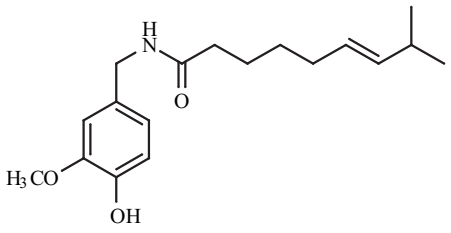
2.2. Synthetic Cannabinoids

Based on all the above results regarding the antiproliferative action of some of the naturally occurring cannabinoids, the next step was to try to emulate these effects but with improved levels of potency, selectivity and control of side effects. With this objective, two of them constitute the main lines of research under current evaluation: (i) design and synthesis of direct agonists of cannabinoid receptors based either on the well known templates of THC or AEA or on completely different structures and (ii) development of compounds able to enhance the physiological endogenous cannabinoid tone, the so-called indirect agonists such as the AEA uptake inhibitors.

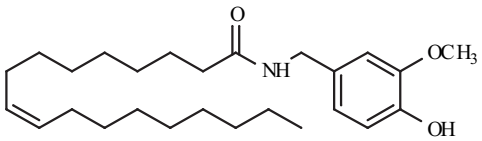
2.2.1. Compounds Structurally Based on the Scaffold of Naturally Occurring Cannabinoids

2.2.1.1. Compounds Structurally Based on the THC Backbone

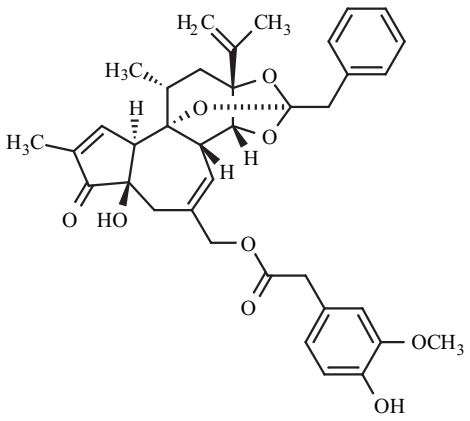
The THC backbone has been the first and the most widely used in an attempt to obtain new cannabinoid compounds able to mimic its effects but deprived of its



Capsaicin



Olvanil



Resiniferatoxin

Fig. (6). Structure of representative vanilloids.

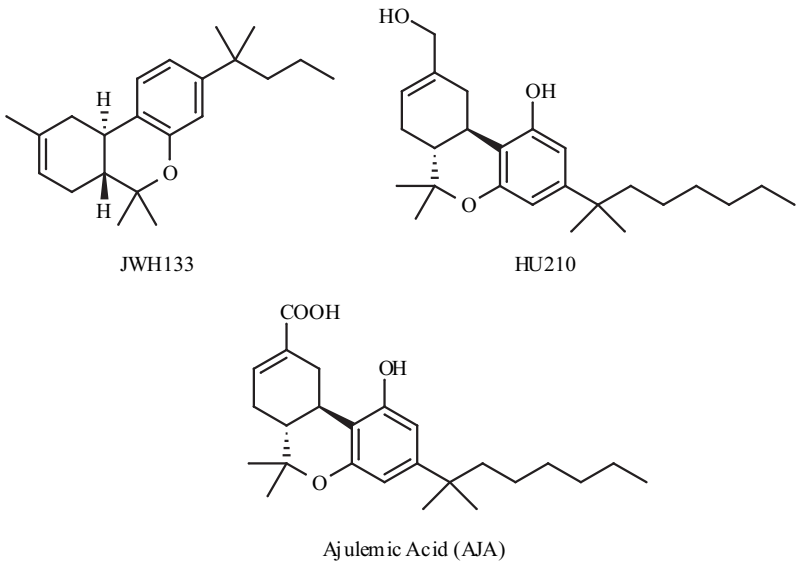


Fig. (7). Structure of synthetic cannabinoids based on THC scaffold.

undesirable properties. Among this class of compounds the most important (Fig. **7**) are the potent but not specific cannabinoid agonist HU210 as well as the CB_2 selective ligands JWH133 and ajulemic acid (AJA).

The antitumoral action displayed by HU210 supports the specific involvement of the cannabinoid receptors in the proliferative control, at least in PC-12 cells and in HBCCs. In PC-12 cells the administration of HU211, enantiomer of HU210 devoid of cannabinoid activity, did not induce any appreciable effect [27]. In HBCCs the inhibition of cell proliferation seems to be CB_1 mediated [18, 19]. In this line, the fact that the apoptotic effect of THC in C6 glioma cells was reversed only with the simultaneous administration of the two cannabinoid antagonists SR141716A and SR144528 [13] also stresses the importance of both cannabinoid receptors in the control of cell proliferation.

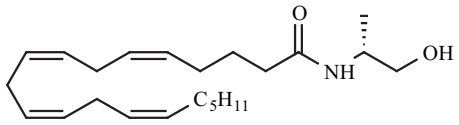
In spite of all these facts, recent evidence seems to indicate that the CB_2 receptor could play a predominant role at least in several types of tumors. In keeping with this, administration of JWH133, a selective CB_2 agonist, to mice in which malignant tumors were generated by inoculation of C6 glioma cells, has allowed not only to inhibit the tumoral proliferation in a notable manner but also to suggest that CB_2 may be a potential marker for the malignancy of astrocytomas since a strong correlation between the CB_2 expression and the degree of tumor malignancy has been recently reported [28].

Closely related to THC, stands out as another antiproliferative agent, the ajulemic acid. AJA is a side-chain synthetic analog of THC-11-oic acid, one of the metabolites originated from THC. AJA has been able to inhibit the tumor growth of different neoplastic cell lines in a specific CB_2-dependent manner, as indicated by the fact that its effects were reversed only in the presence of a CB_2 selective antagonist [29]. Also, and in spite of AJA resulted to be less potent than THC, its antiproliferative effects lasted longer, it showed a very favourable toxicity profile and it was devoid of any psychoactive effects, features that considered together make this compound potentially valuable for chronic use [29], although further studies are still required in order to determine its optimal role as an antiproliferative agent.

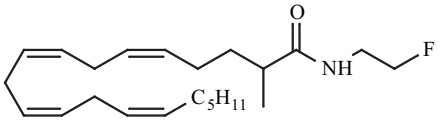
2.2.1.2. Compounds Structurally Based on the Endocannabinoid Backbone

Considering the promising antitumoral properties previously described for endocannabinoids, further efforts have been carried out in order to cope with one of their main associated drawbacks, thus is, their low metabolic stability and, consequently, their short half life. Therefore, it has been of interest to develop new compounds structurally similar to endocannabinoids but more resistant to hydrolytic cleavage.

Among these compounds, (*R*)-methanandamide and (±)-2-methylarachidonyl-2´-fluoroethylamide (Fig. **8**), metabolically stable AEA analogues, have shown remarkable antitumor properties [18, 19, 30] and therefore they constitute promising candidates for further development.



(*R*)-Methanandamide



(±)-2-Methylarachidonyl-2'-fluoroethylamide

Fig. (8). Structure of stable analogues of AEA.

2.2.2. Compounds Structurally Different from the Naturally Occurring Cannabinoids

In the search of more potent and selective cannabinoids a broad variety of compounds, structurally different from THC, have been developed these last years. Among these, some of the most potent ones have also been studied as potential antitumor agents with the aim of providing new

drugs with an optimised profile between desirable and undesirable effects. Within these studies some remarkable results have been reported for JWH105, a CB_2 selective agonist, as well as for CP55940 and WIN552122 (Fig **9**), potent synthetic agonists of both cannabinoid receptors that therefore could be used at lower doses than THC. The two latter were able to induce apoptosis in C6.9 glioma cells with lower IC_{50} values than THC (IC_{50} (CP55940) = 45 nM; IC_{50} (WIN552122) = 20 nM; IC_{50} (THC) = 480 nM) as expected from their higher affinity for cannabinoid receptors [13]. Additionally, WIN552122 showed a potent and direct antitumoral action being able to induce the regression of malignant gliomas in a series of *in vivo* studies without producing any toxic effect [13].

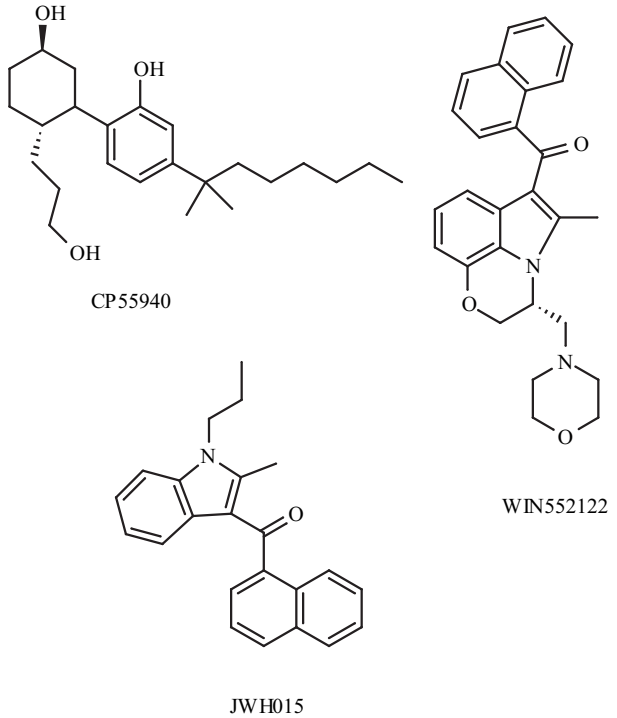


Fig. (9). Representative synthetic cannabinoids structurally different from THC.

In spite of this, the beneficial effects of WIN552122 for the management of cancer are not only limited to its direct antiproliferative action. Very recently has been reported the ability of this compound to produce antihyperalgesia in an *in vivo* murine model of cancer pain, effect partially blocked by the CB_1 antagonist but not by the CB_2 antagonist, suggesting a differential involvement of the two receptor cannabinoid subtypes in this model of deep tissue pain [31].

2.2.3. Indirect Agonists

In an attempt to overcome the disadvantages associated to the administration of cannabinoid agonists, the use of the so-called indirect agonists, thus is, selective inhibitors of the endocannabinoid inactivation, has been suggested. These compounds with no direct action on cannabinoid receptors, even if administered systemically, would not induce any appreciable pschycotropic effects and only would act, in a specific way, in those tissues where the levels of endocannabinoids were pathologically altered. To date, some initial results point to the involvement of endocannabinoids in the regulation of different protein kinases and nuclear factors involved in cancer-cell focal adhesion and migration (see [32] and references herein cited). However, and although this hypothesis constitutes one of the most promising therapeutic approaches and also one of the most active lines of current research, it should be considered as merely theoretical until solid evidences regarding the capacity of endocannabinoids to inhibit tumor growth *in vivo* be obtained.

In spite of all of these considerations, if this hypothesis turns out to be finally of therapeutic value, some of the most valuable candidates to become useful drugs are the most potent inhibitors of endocannabinoid inactivation develop to date. These compounds include the octadecanesulfonyl fluoride (AM381, (Fig. **10**)), which stands out as one of the most potent irreversible inhibitors of FAAH activity (IC_{50} (AM381) = 4 nM) [33], together with the most potent inhibitors of the anandamide transporter (ANT) such as UCM707 (IC_{50} = 0.8 μM) [34, 35], AM404 (IC_{50} = 2.2 μM) [36], and OMDM-1 (IC_{50} = 2.4 μM) [37]. These compounds (Fig. **10**) exhibit different degrees of selectivity for the ANT versus other targets including both cannabinoid receptors, FAAH and VR_1, being the most selective ones UCM707 and OMDM-1 (for a recent review regarding the current status and more relevant features of the ANT inhibitors, see [38]).

From all the above considerations, cannabinoids have turned out to be not only promising antitumoral agents but also valuable drugs for the treatment of cancer related

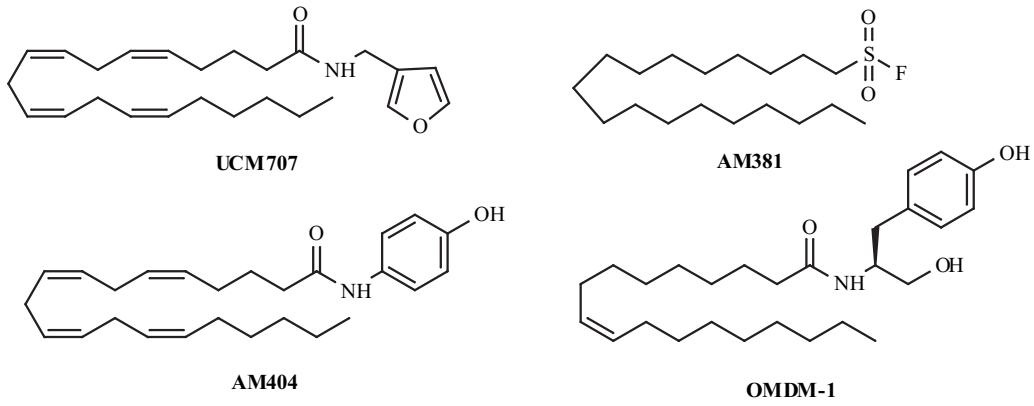


Fig. (10). Representative inhibitors of the inactivation of endocannabinoids.

disorders such as emesis or nociception among others [9, 31]. These evidences about the antiproliferative effects of cannabinoids represent only the starting point when trying to answer the definitive question whether cannabinoids constitute or not a real alternative to face with tumoral processes. The following step is to find out the mechanisms by which cannabinoids produce their effects in order to obtain deeper insights in the physiological and pathological pathways involved in these processes, findings which will eventually allow to develop improved therapeutic strategies for cancer treatment. To date, some of the mechanisms underlying the regulation of the cellular proliferation by cannabinoids have been at least partially clarified but since diverse aspects still need deeper research, this field represents one of the most active lines of current research within the area of cannabinoids. Below, we discuss the current knowledge about the regulatory mechanisms of cell growth by cannabinoids in different cell types providing the most important evidences together with the most widely accepted hypothesis in this field at the present moment.

3. REGULATION OF DIFFERENT CELL TYPES PROLIFERATION BY CANNABINOIDS

3.1. Neural Cells

Cannabinoid agonists have been shown to induce the death of some transformed neural cells while exerting a protective action in normal neurones. Various cannabinoids, specially anandamide and THC, promote apoptosis of astrocytoma, glioma, neuroblastoma, and pheochromocytoma cells in culture by a pathway involving cannabinoid receptors [reviewed in 3, 39]. Inhibition of glioma C6 cell proliferation and human neuroblastoma CHP-100 cells by anandamide may also be mediated by vanilloid receptors [40, 41]. THC and synthetic cannabinoid agonists when administered intratumourally, induce the regression of malignant gliomas in mice and rats [13]. Regression of malignant gliomas has also been seen with the CB_2 receptor-selective agonist JWH133 suggesting that the antitumoral action of cannabinoids on gliomas may be exerted either via CB_1 or via CB_2 receptors [28]. This observation together with the finding that CB_2 expression is enhanced in high grade malignant astrocytomas, provide the bases for therapeutic strategies devoid of the psychotropic side-effects of THC [28]. In this sense, it has been recently reported that AJA, a synthetic non-psychoactive cannabinoid agonist, inhibited the growth of various neoplastic cell lines and the growth of implanted gliomas in mice via CB_2 receptors [29].

By other hand, the endocannabinoid system seems to exert protection in neuronal cells when stimulated with an exogenous insult [42]. Administration of 2-AG to mice after brain injury reduces brain oedema, and hippocampal cell death and improve clinical recovery in a dose-dependent manner and via CB_1 receptor [43]. A protective role of cannabinoid receptors against apoptosis induced by anandamide [40] or ceramide [44] has also been shown. Moreover, the cannabinoid agonist WIN552122 protects C6 glioma cells from citotoxicity induced by the human immuno-deficiency virus-1 Tat protein by a CB_1 receptor-dependent inhibition of the inducible nitric oxide synthase expression [45].

3.2. Immune Cells

Cannabinoids exert very complex effects on the immune system with an influence in almost every immune cell type. Cannabinoids have deleterious effect on many functions of the immune response leading to a general immunosuppressive action. Depending on cannabinoid concentration, cell type and experimental conditions, cannabinoids may inhibit or enhance immune cells growth. Twenty years ago, THC at 10^{-4} M concentration was found to inhibit DNA synthesis in leukaemia cells [46]. Anandamide as well as THC at 10^{-5} – 10^{-4} M have been shown to inhibit lymphocyte proliferation and to cause apoptosis of human blood mononuclear cells [47, 48] and macrophages [49] with no evidence of cannabinoid receptor involvement. On the other hand, THC at 10^{-5} M or greater concentrations, reduces the proliferative response to mitogens and induces apoptosis in thymocytes and splenocytes through the participation of CB_2 cannabinoid receptor [50]. These findings prompted to study whether CB_2 agonists, which lack psychotropic effects, could be used to treat tumors of immune origin [51]. Authors have found that THC, HU210, anandamide and the CB_2 specific agonist JWH015, significantly reduced the cell viability of immune tumor cells as well as *in vivo* tumor growth in tumor-bearing mice [51]. This effect is mediated at least in part by CB_2 receptors because the CB_2 antagonist SR144528 partially blocks the effect induced by cannabinoids [51].

By contrast, low doses of THC, WIN552122 and CP55940, induce a dose-dependent increase in human B lymphocyte proliferation that is inhibited by pertussis toxin, suggesting a receptor-mediated mechanism [52]. However, in murine lymphoid cell lines, anandamide activates cell growth by a cannabinoid receptor-independent way [53]. Anandamide at 10^{-7} M and 10^{-6} M concentration may also induce the growth of hematopoietic cells in which may act as a natural synergistic growth factor enhancing the proliferative response of cells to hematopoietic growth factor through activation of CB_2 [54]. Up-regulation of CB_2 receptors in lymphomas has been observed using micro-array chips [55] and increasing CB_2 mRNA levels have been detected in myeloid leukaemia cells [56]. In murine leukaemia cells, activation of CB_2 with 2-AG stimulates cell migration [56] and blocks neutrophilic differentiation [57]. All these data provide the bases to consider the CB_2 as a novel protooncogene [58].

3.3. Endocrine and Exocrine Cells

The expression of the CB_1 cannabinoid receptor as well as the levels of the endocannabinoids AEA and 2-AG, in normal and tumoral pituitaries have been studied [59]. All tumoral samples had higher contents of AEA and 2-AG compared with the normal hypophysis [59]. The endocannabinoid content in pituitary adenomas correlated with the presence of CB_1, being elevated in the tumoral samples positive for CB_1 [59]. Cannabinoids induced modulation of hormone release in pituitary tumors [59] as well as in pituitary tumor cell lines [60].

Studies performed by Di Marzo *et al*. demonstrated that the metabolically stable anandamide analogue, (±)-2-methylarachidonyl-2′-fluoroethylamide, inhibited the *ras*

oncogene-dependent growth of a thyroid tumor *in vivo* and *in vitro* [30]. The anti-tumor effect induced by the anandamide analogue was abolished by the CB_1 antagonist SR141716A, indicating that it was exerted through the cannabinoid receptor [30]. Moreover, when K-*ras*-transformed thyroid cells were treated with the anandamide analogue, an up-regulation of the CB_1 cannabinoid receptor was observed [30]. (±)-2-methylarachidonyl-2′-fluoroethylamide not only inhibited tumor growth *in vivo* but also the expression of the angiogenic factor vascular endothelial growth factor (VEGF) and one of its receptors [61]. The growth inhibitory effect induced by the anandamide analogue was more prominent in metastatic cells than in a primary cancer cell line [61]. These findings point to cannabinoid receptors and endogenous cannabinoids as potential therapeutic targets to control tumor growth.

Cannabinoid agonists may inhibit the growth of breast cancer cell lines in a dose-dependent manner. AEA and its stable analogue (*R*)-methanandamide as well as 2-AG and the synthetic cannabinoid HU210, have a cytostatic effect in human breast epitheloid EFM-19 cells, which stop at S phase of the cell cycle [18]. AEA also inhibits the proliferation of human breast MCF-7 cells by interaction with prolactin action. The effect of AEA is mediated by the suppression of the prolactin receptor level acting via a mechanism dependent on the cannabinoid receptor CB_1 [18]. The same authors demonstrated that AEA inhibits nerve growth factor (NGF)-induced proliferation of human breast cancer cells by decreasing the levels of the high affinity *trk* NGF receptor through a CB_1 receptor-dependent pathway [19]. The inhibitory effect of AEA is prevented by forskolin and inhibitors of the MAPK (mitogen activated protein kinase) pathway suggesting that anandamide suppresses NGF receptors and thereby cell proliferation, with the activation of the MAPK cascade and inhibition of adenylyl cyclase [62]. The anti-proliferative effect of AEA in breast cancer cells may be enhanced when cells are incubated with inhibitors of AEA degradation pointing to new targets for pharmacological therapies [22, 26].

Cannabinoids may also exert a modulatory effect on the proliferation of prostate cancer cells. AEA suppresses the expression of NGF and epidermal growth factor (EGF) receptors in prostate cancer cells and by this mechanism inhibits prostate cell proliferation [19, 63]. The suppression of growth factor receptor levels is mediated by the CB_1 receptor expressed in these cells, as it may be blocked by the receptor antagonist SR141716A [19, 26]. Long-term treatment with cannabinoid agonists also induces apoptosis of prostate cancer cells but in this case the effect may not be inhibited by the CB_1 antagonist SR141716A [63, 14].

Thus, cannabinoid agonists seem to inhibit prostate cancer cell proliferation through several mechanisms. Massive apoptosis induced at long-term treatment with micromolar doses of cannabinoids seems to be not necessarily mediated by CB_1 receptors [63, 14] whereas inhibition of growth factor- or hormone-stimulated cellular growth is mediated by CB_1 receptors [19, 63]. The response of glandular cells to growth factors is specially relevant in pathological conditions. Breast cancers and prostate cancers are among the most frequent metastatic malignances that cause cancer death in females and males respectively. Tumor development of these glands depends on the deregulated balance between growth factors and circulatory hormones. Indeed, one of the main therapeutic treatment developed to date is the pharmacological or surgical steroid hormones ablation to block the malignant growth. In the case of prostate cancer, although tumour cells respond initially to androgen ablation therapy, most tumours eventually recur in an androgen refractory manner [64]. In this case, malignant cells growth may be enhanced by growth factors and cytokines [65], and at the present there is no successful treatment for this refractory tumours. The suppression of growth factor receptor levels by cannabinoids in prostate malignant cells is very promising since it may allow the development of new therapies based on cannabinoids.

4. MECHANISMS OF CANNABINOID ACTION

4.1. Ceramide Generation

The mechanisms that underlie the antineoplasic activity of cannabinoids are not well understood. Recent findings suggest that ceramide production is an important step in the apoptosis induction by cannabinoids [66]. Ceramide is a sphingolipid messenger that is almost universally generated during cellular stress and apoptosis [67]. Exposure of glioma cells to cannabinoids triggers the generation of two peaks of ceramide, being the second peak that starts at 3 days of treatment, related with the apoptotic death of glioma cells [13]. It has been recently demonstrated that the mechanisms whereby cannabinoids generate the sustained ceramide increase involves *de novo* synthesis of ceramide and the activation of serine palmitoyltransferase, which catalyses the rate limiting step of ceramide synthesis [64, 68]. Involvement of ceramide generation has also been demonstrated in prostate cells, in which the inhibition of cell proliferation by anandamide could be blocked by the specific ceramide synthetase inhibitor fumonisin B1 [63]. *De novo* ceramide biosynthesis has been implicated in the apoptosis induction by anticancer drugs [69] and a slow and sustained ceramide elevation has been found in the effector phase of death stimuli [70]. This ceramide production may facilitate membrane blebbing, vesicle shedding and apoptotic body formation [71].

4.2. Activation of Caspases

Apoptotic death is regulated by caspases, a set of cysteine proteases with specificity for aspartic acid residues, that become active during apoptosis [72]. Caspases have been divided into initiator caspases that can be activated by protein-protein interaction and executioner caspases that are activated by proteolytic cleavage by other caspases. Cannabinoid agonists may activate the major executioner caspase-3 in some cells. Anandamide induces apoptosis of the rat pheochromocytoma PC-12 cells and the activation of caspase-3 measured by its protease activity, that may be prevented with the antioxidant *N*-acetylcysteine [73]. On the other hand, the apoptotic death induced by THC in cortical neurones is accompanied by the activation of caspase-3 by a CB_1 receptor-dependent mechanism [74]. However, AEA failed to induce apoptosis in MCF-7 cells [18, 73] and this may be attributed to the caspase-3 deficiency in this cell line [75]. Activation of caspase-3 by cannabinoids may cleave the

inhibitor of caspases-activated Dnase (iCAD) releasing the caspases-activated Dnase (CAD), which in turn cuts the chromatin between nucleosomes to produce the DNA ladder seen in apoptotic cells. The activation of caspase-3 by cannabinoids seems to occur by the mitochondrial pathway since release of mitochondrial cytochrome C to the cytosol has been observed in cortical neurones stimulated with THC [74] and regulation of Bcl-2 mRNA and protein is induced by THC in splenocytes [73].

5. CLINICAL STUDIES

Epidemiological studies about marijuana smoking and the risk or the prevention of cancer are limited and the results are controversial. The first question is to separate the effect of THC or cannabinoids from the carcinogenic effect induced by the pyrolysis products of marijuana tar. By other hand, in marijuana there are multiple substances that may work synergistically, additively or antagonistically when ingested together by smoking. Cannabis smoking is suspected to increase the risk of cancer of the aerodigestive tract and possibly of lung at least comparable to that of tobacco smoking [76, 77] and airway biopsies obtained from marijuana smokers showed precancerous histopathologic alterations similar to those observed in tobacco smokers [78]. Moreover, it has been recently demonstrated that marijuana tar extracts and THC enhanced the levels of carcinogen-metabolising enzyme CYP1A1 mRNA, which is a key step in the development of tobacco-related cancers [79]. By other hand, several publications have recently suggested that cannabis use may contribute to the development of neck and head cancer [80, 81]. However, in a previous study reported by Sidney *et al*. [82] with 64855 participants, the use of marijuana was not associated with increased risk of tobacco-related cancers. Such discrepancies may be due to differences in the age of the examinees or the time cannabis use, since respiratory tract cancers begin to increase over the age of 60 years.

This is not the case for endocrine-related tumors. In the same study by Sidney *et al*., males who had smoked cannabis and never smoked tobacco, had an increased risk of prostate cancer [82]. Cannabis use was also associated with increased risk of cervical cancer in women [82]. An increase in the development of endocrine tumors has been also observed in rats treated with marijuana after gamma irradiation [83].

There are few published epidemiological studies about the action of THC or isolated cannabinoids on human cancer development. Clinical studies of THC as antitumoral agent are currently underway. Anti-cancerous effects of THC and other cannabinoids have been demonstrated *in vivo* in experimental animals [8, 13, 28, 29]. In mice and rats, chronic administration of THC did not produced increase of neoplasms and reduced the rate of spontaneous tumors commonly found in rats and mice [84]. Inhibition of proliferation is not the only mechanism whereby cannabinoid agonists block tumour progression *in vivo*. Recent studies demonstrate that cannabinoids inhibit tumor angiogenesis *in vivo* determined by altered blood vessel morphology and decreased expression of angiogenic factors [61, 85, 86]. These observations provide new strategies for the use of cannabinoids as antitumoral agents. However, it should be taken into consideration that cannabinoids exert their actions in several tissues and the overall biological action results from the interaction between many systemic factors. As cannabis exert an immunosuppressive effect in the immune system, marijuana smoking or long-term cannabinoid treatment may suppress host immune reactivity against some tumor cells that may be unnoticed in experiments with nude mice *in vivo*. In a recent study with immunocompetent mice, administration of THC led to accelerated growth of lung cancer compared to controls whereas THC did not affect the tumor growth on nude mice [87]. The inhibition of antitumor activity by THC was mediated by the CB_2 cannabinoid receptor [87].

6. FUTURE PERSPECTIVES

Our molecular understanding of the involvement of cannabinoids in the regulation of the cellular proliferation has experimented a significant progress during the last years due to the parallel efforts carried out by biochemists and synthetic chemists. The simultaneous development of both lines of research has therefore provided with synthetic agents ready to be used as valuable tools for the elucidation of some of the underlying pathways involved in the mechanism of action of cannabinoids.

These tools, within basic research, together with the different animal models developed, as clinical research, suggest that the ECS may be a promising therapeutic target for the treatment of tumoral processes. However, further research is still needed in order to obtain a definitive answer regarding the actual potential of cannabinoids to treat cancer and whether these compounds are actually more effective and safe than other antitumoral drugs currently available.

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LIST OF ABBREVIATIONS

AEA = *N*-Arachidonoylethanolamine, anandamide

AJA = 1',1'-Dimethylheptyl-Δ^8-THC-11-oic acid, ajulemic acid

2-AG = 2-Arachidonoylglycerol

ANT = Anandamide transporter

BSA = Bovine serum albumin

CAD = Caspases-activated Dnase

CB_1 = Cannabinoid receptor subtype 1

CB_2 = Cannabinoid receptor subtype 2

CNS = Central nervous system

ECS = Endogenous cannabinoid system

EGF = Epidermal growth factor

FAAH = Fatty acid amidohydrolase

HBCCs = Human breast cancer cells

iCAD = Inhibitor of caspases-activated Dnase

IC_{50} = Compound concentration which causes a 50% of inhibition in the target considered

K_i = Affinity constant

Max. Stim = Maximum stimulation

MAPK = Mitogen activated protein kinase

NGF = Nerve growth factor

NO = Nitric oxide

OEA = Oleoylethanolamide

PEA = Palmitoylethanolamide

SAFIR = Structure-affinity relationship

trk NGF = High affinity NGF receptor

VEGF = Vascular endothelial growth factor

VR_1 = Vanilloid receptor subtype 1

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