Cannabinoids and Cancer

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Abstract: Marijuana has been used in medicine for millennia, but it was not until 1964 that Δ9-tetrahydrocannabinol (Δ^9-THC), its major psychoactive component, was isolated in pure form and its structure was elucidated. Shortly thereafter it was synthesized and became readily available. However, it took another decade until the first report on its antineoplastic activity appeared. In 1975, Munson discovered that cannabinoids suppress Lewis lung carcinoma cell growth. The mechanism of this action was shown to be inhibition of DNA synthesis. Antiproliferative action on some other cancer cells was also found. In spite of the promising results from these early studies, further investigations in this area were not reported until a few years ago, when almost simultaneously two groups initiated research on the antiproliferative effects of cannabinoids on cancer cells: Di Marzo's group found that cannabinoids inhibit breast cancer cell proliferation, and Guzman's group found that cannabinoids inhibit the growth of C6 glioma cell. Other groups also started work in this field, and today, a wide array of cancer cell lines that are affected is known, and some mechanisms involved have been elucidated.

Keywords: Cannabinoid, endocannabinoid, mechanisms, cancer, angiogenesis.

INTRODUCTION

Marijuana has been used in medicine for many centuries, but it was not until 1964 that Δ^9-tetrahydrocannabinol (Δ^9-THC), it's major psychoactive component, was isolated in pure form and its structure was elucidated (see Fig. **3** for cannabinoids' structures) [1]. Shortly thereafter it was synthesized and became widely available. These chemical advances led to an avalanche of publications and thousands of reports appeared on Δ^9-THC [2], as well as on other *Cannabis sativa* constituents. It is now known that cannabinoids exert a wide array of effects on the immune [3,4], digestive [5], reproductive [6,7] ocular [8], cardio-vascular [9,10] and central nervous systems [11-13].

The first report on antineoplastic activity of cannabinoids appeared in 1975, when Munson discovered that cannabinoids inhibit Lewis lung carcinoma growth [14], through blockage of DNA synthesis in these cells [15,16]. Cannabinoids also increased the life span in mice carrying Lewis lung carcinoma and decreased primary tumor size [17]. Soon afterwards, other cancer cell lines were found to be affected by cannabinoids. Thus, Δ^9-THC inhibited DNA, RNA and protein syntheses in L1210 murine leukemia cells [18]. It also attenuated the proliferation of K-562 leukemic cell line [19]. The mechanism by which Δ^9-THC inhibited B103 neuroblastoma cell proliferation was quite different – it caused dose-related alterations in cell morphology, that included rounding of cells, retraction of neurites, changes of cell surface, exfoliation of plasma membrane, distention of endoplasmic reticulum, Golgi apparatus and perinuclear space, macrovacuolization, and cytoskeletal rearrangement [20]. In 1987, Huberman found that cannabinoids induce incomplete maturation of human myeloblastic ML-2 leukemia cells [21]; the same effect was observed also in a HL60 cell line [22].

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In spite of the promising results from the early studies, further investigations were not undertaken in this area until a few years ago, when almost simultaneously two groups initiated research on the antiproliferative effects of cannabinoids on cancer cells: Di Marzo's group found that cannabinoids inhibit breast cancer cell proliferation [46](Jul 1998), and Guzman's group found that cannabinoids inhibit the growth of C6 glioma cell [23] (Sep 1998).

Nowadays, a wide array of cancer cell lines that are affected by cannabinoids is known, and some of the mechanisms have been unraveled.

CANNABINOID EFFECTS ON GLIOMA CELLS AND THEIR MECHANISMS

The effects of cannabinoids on glioma cells have been more thoroughly investigated than in other transformed cells.

The first report on the action of cannabinoids on C6 glioma cells was in 1998, when Guzman's group discovered that Δ^9-THC (Fig. **3**) induces apoptosis in these cells [23]. It was shown that the action of THC was not directly mediated through binding to cannabinoid receptors, but apparently was caused by ceramide, a product of sphingomyelin breakdown.

Ceramide is a ubiquitous lipid second messenger that plays an important role in the control of cell fate [24]. It is involved in the induction of apoptosis. THC induced a considerable accumulation of ceramide and caused activation of Raf1, a pivotal element in the control of cell fate by the ERK cascade. It was shown that the Raf1 activation was not caused by Ras, a protein kinase that usually leads to this action, but probably by ceramide, which may directly bind to and activate Raf1 [25]. Thus, the apoptotic action of THC seems to depend on long term activation of the ERK cascade, which in this process mediates cell differentiation and anti-proliferation [26,27] rather than proliferation (the

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usual action of ERK cascade [28]). The long-term ceramide accumulation, leading to cannabinoid-induced apoptosis, was sustained through enhanced synthesis *de-novo* [29], rather than by acute ceramide generation. None of sphingomyelinase inhibitors prevented THC-induced death in C6.9 glioma cells. In contrast, blockage of ceramide synthesis *de-novo* prevented THC-induced death, abolished THC-induced stimulation of ERK and inhibition of PKB [30]. Although some cannabinoids (such as THC and WIN-55,212-2) (Fig. **3**) bind to both cannabinoid receptors, [25,31] the selective activation of CB2 cannabinoid receptor alone also causes inhibition of glioma growth *in vivo* and *in vitro* [31]. Apparently, cannabinoids cause ceramide synthesis *de-novo* and ERK activation [31] through CB2 activation. However, a further mechanism exists through which cannabinoids elevate ceramide levels in glioma cells. Met-AEA elevates ceramide levels in human neuroglioma cells [98]. This enhancement leads to MAPK induction and COX-2 generation [98], which causes apoptosis in these cells [115]. The pro-apoptotic effect is prevented by the COX-2 inhibitor celecoxib [115]. The effect is not mediated by CB1/CB2/VR1 receptors [98], but probably is mediated by membrane lipid rafts, as methyl β-cyclodextrin (MCD), which causes depletion of membrane cholesterol, inhibits Met-AEA induced COX-2 expression and subsequent formation of PGE2 [113].

Cannabinoids, as well as C2-ceramide (a cell permeable ceramide analog) inhibit VEGF production in C6 glioma cells. The lower levels of VEGF diminish angiogenesis and pro-survival VEGF signals, thus causing loss in cancer cell viability [106].

Cannabinoid Actions on Glioma Cells that are not Through the Ceramide Pathway

A recent report discloses that WIN-55,212-2, a CB1/CB2 receptor agonist, inhibits C6 glioma cell proliferation by inhibition of ERK and PKB/Akt pro-survival pathways and attenuation of phosphorylation and decomposition of Bad, a small pro-apoptotic member of Bcl-2 family [107]. This report contradicts other studies that show that activation of ERK cascade by cannabinoids lead to cell death and ceramide *de-novo* synthesis [25,30,31]. It also contradicts studies that show cannabinoid-mediated PI3K elevation, that mediate protective/proliferative role of cannabinoids [53,86,92].

Anandamide (Fig. **3**), the first endocannabinoid to be identified, is a partial agonist of both CB1 and CB2 receptors [32-34], and also of vanilloid receptors [34]. In some human cancer cell lines, anandamide's pro-apoptotic effects are mediated through vanilloid receptors (see below). Some of the effects of anandamide on C6 glioma cells are not mediated by CB1 and CB2 receptors and the ceramide pathway. Neither CB1 nor CB2 selective antagonists, alone or together, block its action. However, when capsazepine (a vanilloid receptor antagonist) is administered together with the antagonists of CB1 and CB2, the effects of anandamide are completely blocked [35]. A remarkable feature is that oxidative stress and excessive intracellular calcium are involved in the antiproliferative effect of anandamide, as it can be completely blocked by α-tocopherol (an antioxidant) and calpeptin (a calpain inhibitor) [35]. In another study, AEA was able to cause C6 cell apoptosis mainly through VR1, while CB1 receptor protected the cells from VR1-mediated damage (as CB1 antagonist increased this damage) [53]. In U87, U251, Ge227 and Ge258 human glioma cell lines, AEA causes cell apoptosis partially through VR1, as capsazepine, a VR1 receptor antagonist, partially inhibits its effect; here also cannabinoid receptors play a protective role [112]. However, straightforward conclusions cannot be drawn from these studies, as the inhibition of C6 glioma cell proliferation by Met-AEA, a stable analog of AEA, was not prevented by the joint action of CB1, CB2 and VR1 antagonists. This observation, together with the finding that arachidonic acid also inhibits the proliferation of these cells suggests that either AEA and Met-AEA mechanisms of action differ or that AEA perhaps acts through its hydrolysis product arachidonic acid. As blockage of fatty acid amide hydrolase (FAAH), the enzyme involved in the AEA's degradation to arachidonic acid did not reduce the effect of AEA, it seems that a novel mechanism of action is presumably involved [90].

The effect of 2-AG and 1-AG on C6 glioma cells has also been assayed. 2-AG and 1-AG inhibited C6 glioma cell proliferation with very similar potencies to that of AEA, suggesting that 2-AG exerts its antiproliferative effects by a mechanism parallel to that of AEA. The combination of capsazepin with CB1 and CB2 antagonists blocked the effect of 2-AG and 1-AG, but SB366791, a selective VR1 antagonist, was less effective than capsazepin, suggesting that a VR1-independent mechanism is also involved [90].

CBD (Fig. **3**), a non-psychotropic cannabinoid, is also capable of killing glioma cells, by causing apoptosis. Curiously, its effects are prevented neither by CB1 and VR1 receptor antagonists nor by the inhibitors of ceramide synthesis, but are partially prevented by a CB2 receptor antagonist and α-tocopherol, suggesting oxidative stress involvement [91], as also seen with other cannabinoids.

AM404 and VDM11 inhibit anandamide transport into the cell and thus, also inhibit its cellular accumulation, causing potentiation of its extracellular actions [83,84]. Surprisingly, they inhibited C6 cell proliferation by a non CB1/CB2/vanilloid mechanism, as their action was prevented neither by cannabinoid nor by vanilloid receptor antagonists. The antioxidant α-tocopherol, which, as mentioned above, prevents death induced by anandamide [35], likewise had no effect [85]. However, in a recent study it was shown that in thyroid epithelioma cells, the CB1 antagonist SR141716A (Fig. **3**) counteracts VDM11's effect and, though to a smaller extent, the effects of 2-AG and AA-5-HT (a selective anandamide hydrolysis inhibitor) [108].

Stearoylethanolamide (SEA) (Fig. **3**) is an endo-cannabinoid-like compound found in the brain of mammals, in even higher amounts than anandamide. SEA induces apoptosis in C6 glioma cells, although it does not bind to CB1, CB2 and only partly binds to vanilloid receptors. SEA binds to some other specific site on the C6 cell membrane, as cannabinoid receptors agonists have an additive effect with SEA in causing apoptosis [36]. The SEA-caused apoptosis proceeds through activation of cyclooxygenase and lipoxygenase, and does not involve the MAPK and PI3K cascades. The result is mitochondrial

uncoupling, increase in intracellular calcium concentrations and apoptotic body formation [36].

The action of ajulemic acid (a psychotropic synthetic cannabinoid, which binds to both CB1 and CB2 receptors) (Fig. **3**) on C6 cells is quite unexpected. It does not lead to apoptosis and does not affect cell cycle kinetics; it causes the appearance of refractile bodies that are stained with red O stain, which is specific for TGs and cholesteryl oleate [37]. Ajulemic acid-treated cells were enlarged and contained large lipid droplets; its effect was cytostatic rather than cytotoxic. The effect of ajulemic acid was mediated by CB2 cannabinoid receptors [37].

The contribution of CB1 *vs.* CB2 receptors in the antiproliferative effect on glioma cells remains unclear. In some cases, cannabinoids act through both receptors [25,31], in others, through CB2 alone [31,37,91]. Both receptors seem to activate the ceramide pathway. A recent study shows that CB1 receptor may also activate the JNK pathway through βγ subunits of Gi, which leads to activation of Ras or Rac, upstream of the JNK cascade [38]. JNK in many cases is involved in cell death [39,40,41]. In C6.9 glioma cells, the remarkable induction of JNK activity caused by THC is accompanied by a significant decrease in cell viability [38], thus suggesting a mechanism, different from the ceramide-ERK pathway, by which cannabinoids can kill glioma cells. One more interesting feature is that in some cases cannabinoids can transactivate PDGF receptors, which can then activate JNK. The modulation of this growth factor receptor can represent the pathway by which cannabinoids control cell fate [38]. In some cases however, neither of these receptors is involved (or are involved in part only) and the main action is apparently through VR1 receptors [35,90,112].

CANNABINOID EFFECTS ON GROWTH FACTOR/HORMONE-DEPENDENT (BREAST AND PROSTATE) CANCER CELLS AND THEIR MECHANISMS

As endocannabinoids are known for their stimulatory/suppressing effects on the levels of some hormones [42,43] and suppress the regulatory action of the hypothalamo-pituitary-adrenal axis [44,45], it seemed reasonable to expect that they would have some effect on breast cancer cell proliferation, which depends on prolactin and estrogen. Indeed, anandamide inhibited the proliferation of EFM-19 cells and also of some other breast cancer cell lines, such as MCF-7, T-47D and BT-47446. The antiproliferative effect was due to inhibition of DNA synthesis; anandamide inhibits the G1 to S transition of the cell cycle. The effect is mediated by CB1 receptors.

Cannabinoids (not only anandamide but also other agonists such as HU-210, 2-AG) (Fig. **3**) cause a strong down-modulatory effect on the levels of prolactin receptors in the above cell lines. The antiproliferative potency of cannabinoids parallels the degree of dependency of cell proliferation on prolactin [46]. In breast cancer cells, the NGF receptors *trk* are also down-regulated by cannabinoids, which lead to the inhibition of these cell proliferation [47,48]. The intracellular mechanism involves the inhibition of adenylate cyclase (AC) activity, and lowering of cAMP levels and PKA activity. Cannabinoids also inhibit the prolactin-induced proliferation of DU-145 prostate cancer cells, by down-modulating the prolactin receptors [48]. The effects are through cannabinoid receptors, on the Gi/o protein, and the activation of the ERK pathway (through Raf-1, whose activity is lowered by PKA) [47], which is the most thoroughly investigated pathway of cannabinoid antiproliferative activity. Arvanil effects are partly caused by vanilloid receptors [48].

Another mechanism by which anandamide inhibits the growth of DU-145 and PC3 prostate cancer cells is by decrease of the amount of EGF receptor, which is also mediated by a CB1-AC-PKA pathway, similar to the down-modulation of prolactin receptors. As in breast cancer cells, anandamide inhibits the G1 to S transition in these cells [86]. As mentioned above, anandamide also acts in these cells through *de-novo* synthesized ceramide. LNCaP cells (another prostate cancer cell line) are much less sensitive to growth inhibition effect of anandamide, as EGF has less proliferative effect on these androgen-responsive cells [86]. Furthermore, anandamide's analog methanandamide upregulates the androgen receptor in these cells and is even able to enhance the cell proliferation of these cells. However, the proliferation enhancement is seen only in cells treated with very low (0.1μM) methanandamide concentrations; in the concentrations above 1μM, methanandamide causes cell death [87]. The mechanism of receptor upregulation here is through both cannabinoid receptors CB1 and CB2 and is mediated by the PI3K pathway [87]. CB1 is coupled to the activation of PKB through PI3K, stimulated by βγ subunits of Gi/o [54]. The PKB pathway is known to be one of the important pro-survival pathways, its anti-apoptotic action is mediated by inactivation of Bad, caspase-9 and Forkhead transcription factors[55,56,57]. When PC3 prostate cancer cells are exposed to THC or methanandamide (in low concentrations), their proliferation increases through PKB/Akt activation and NGF induction. The latter is mediated by the activation of ERK1/2 through both cannabinoid receptors [92].

The Potentiation of Cannabinoid Effects

The endocannabinoid-like compound oleamide (a sleep factor) (Fig. **3**) also shows antiproliferative effect on EFM-19 cells, though it is much less potent than anandamide. However, it greatly potentiates anandamide's antiproliferative effect, apparently by minimizing anandamide hydrolysis by FAAH (fatty acid amide hydrolase) [49]. A higher rate of apoptotic death is seen also in PC3, DU-145 and LNCaP prostate cancer cells in response to anandamide + oleamide, than with any of them alone [86].

The action of palmitoyl-ethanolamide (PEA) (Fig. **3**) is more complicated. This compound greatly enhances the antiproliferative and PRL-R and *trk* down-modulatory effect of anandamide on human breast and prostate cancer cell lines [50]. PEA is not a very efficacious inhibitor of AEA hydrolysis by FAAH [51], but it significantly decreases the expression of FAAH at the transcriptional level [50]. PEA also enhances the inhibition of both basal and NGF-induced breast cancer cell proliferation by capsaicin, a vanilloid receptor agonist, possibly by an allosteric effect on VR1

receptors [52]. PEA also induces antiproliferative effects by HU-210 [50], by is yet an unknown mechanism.

OTHER MECHANISMS OF CANNABINOID EFFECTS ON CANCER CELLS

Vanilloid Receptor-Mediated Effects of Cannabinoids on Cancer Cells

Some cannabinoid effects on cancer cells are mediated, at least in part, through vanilloid receptors. The effects of anandamide on C6 glioma cells, breast and prostate cancer cells are partly mediated by these receptors [35,48], but in some cases, the antiproliferative effect of anandamide is caused only by vanilloid receptors. Thus, anandamide causes apoptosis in human neuroblastoma CHP100 cells and human lymphoma U937 cells, through neither of the cannabinoid receptors, but through vanilloid receptors [53]. The intracellular mechanisms are mitochondrial uncoupling, intracellular calcium rise and cytochrome C release, with subsequent activation of caspases 3 and 9 [53].

An intriguing feature is that cannabinoid receptors can in some cases protect cells from the damage caused by vanilloid receptors. As mentioned above in U87, U251, Ge227 and Ge258 human glioma cell lines, AEA causes cell apoptosis partially through VR1, as capsazepine partially inhibits its effect; here the cannabinoid receptors play a protective role [112]. Also in C6 glioma cells and DAUDI leukemia cells, cannabinoid receptors antagonists increase apoptosis caused by anandamide, suggesting some protective role for these receptors [53]. This can be explained by the fact that CB1 is coupled to the activation of PKB through PI3K, stimulated by $\beta\gamma$ subunits of Gi/o [54]. The PKB pathway is one of the important pro-survival pathways; it's anti-apoptotic action is mediated by inactivation of Bad, caspase-9 and Forkhead transcription factors [55,56,57].The same effect on PKB/Akt activation is seen in some other cancer cell lines after treatment with AEA, THC, HU-210 and WIN55,212-2, where it is mediated by TACE's (tumor necrosis factor α converting enzyme) mediated shedding of growth factors that activate EGFR [93] and in PC3 prostate cells where PKB/Akt and NGF are induced through both cannabinoid receptors [92]. In CxCa uterine cervix cancer cells, AEA induced apoptosis is also through VR1 receptor; here also the protective role of cannabinoid receptors CB1 and CB2 can be seen [94].

Action Through Oxidation of Cell Contents

Anandamide induces apoptosis in PC-12 pheochromocytoma cells by generation of intracellular superoxide anion that triggers caspase activation [58]. The antioxidant N-acetyl cysteine prevents this apoptotic death [58]. The importance of anandamide-induced cell death through intracellular oxidation has received additional experimental support. Thus, in C6 cells, antioxidants prevented anandamide, 1-AG, 2-AG, and CBD induced cell death [35,90,91]. Stearoyl ethanolamide likewise induces apoptosis in these cells, which is mediated by oxidation of the contents of the cells [36]. One can conclude that the oxidation pathway is very important, and maybe the main mechanism of endocannabinoid action on cancer cells.

The induction of apoptosis by anandamide in PC-12 cells is modulated by ASK-1 (apoptosis signal regulating kinase 1), which activates JNK and p38 MAPK, that in turn induce cytochrome C release from mitochondria and caspase activation. The cell death in this case is not mediated by CB1 cannabinoid and VR1 vanilloid receptors [88]. In some cases none of the CB1, CB2 or VR receptors are involved in anandamide-caused apoptosis.

A related mechanism seems to be involved in the blocking of anandamide-induced cell death by methyl-beta cyclodextrin (MCD), which causes cholesterol depletion in cell. This effect has been noted in a variety of cells, including PC12, C6, Neuro-2a, CHO, HEK, SMC, Jurkat and HL-60 cells. MCD also blocks anandamide-induced superoxide generation, phosphatidylserine exposure and p38 MAPK/JNK activation. This data imply a novel role for of membrane lipid rafts in anandamide-induced cell death [100,113], as mentioned above as regards neuroglioma cells [115].

Cannabinoid Receptor-Mediated Effects of Cannabinoids on Cancer Cells

The action of Met-F-anandamide (a stable anandamide analog) was investigated in transformed thyroid cells. Met-F-anandamide blocks p21ras action through CB1 receptors and inhibits the growth of KiMol tumors (from *K-ras* transformed FTRL-5 thyroid cells) [59]. Ras proteins (*H-ras, N-ras, K-ras*) are the key molecules in the signal transduction pathways leading to cell proliferation, differentiation or death. The mutation of *K-ras* is most commonly found in human tumors [60]. Met-F-anandamide causes the cell cycle arrest at the G0/G1 phase [59]. This effect was also seen in breast and prostate cancer cells, where anandamide also causes cell cycle arrest [46,47]. In another study, intratumor administration of VDM11 and AA-5-HT, both of which enhance intratumoral endocannabinoids levels, strongly inhibit KiMol tumor growth. The effect was partially counteracted by SR141716A [114]. THC causes apoptosis in malignant lymphoma cells such as Jurkat, Molt-4 and Sup-T1EL-4 cells. The mechanism is unclear, but involves mainly CB2 receptors [63]. It is apparently not through IL-2 downregulation, as it was initially assumed, because cannabinoid receptors are coupled to Gi/o proteins that inhibit AC activity and cAMP levels, and cAMP is known to downregulate the production of IL-2 [64].

Non CB1/CB2 non VR1- Mediated Cannabinoid Effects on Cancer Cells

Cannabinoids play a role in colorectal cancer growth inhibition. Anandamide, 2-AG, and HU-210 potently inhibit CaCo colorectal carcinoma cell line proliferation through CB1 receptor and in DLD-1, colorectal carcinoma through both cannabinoid receptors [89].

Plant cannabinoids also affect cancer cells. Apoptosis in PC-3 prostate cells is induced by Δ^9-THC, but not through the known cannabinoid receptors [61]. Cannabigerol, which has a minimal affinity to cannabinoid receptors, inhibits KB human oral epitheloid carcinoma cell line growth [62].

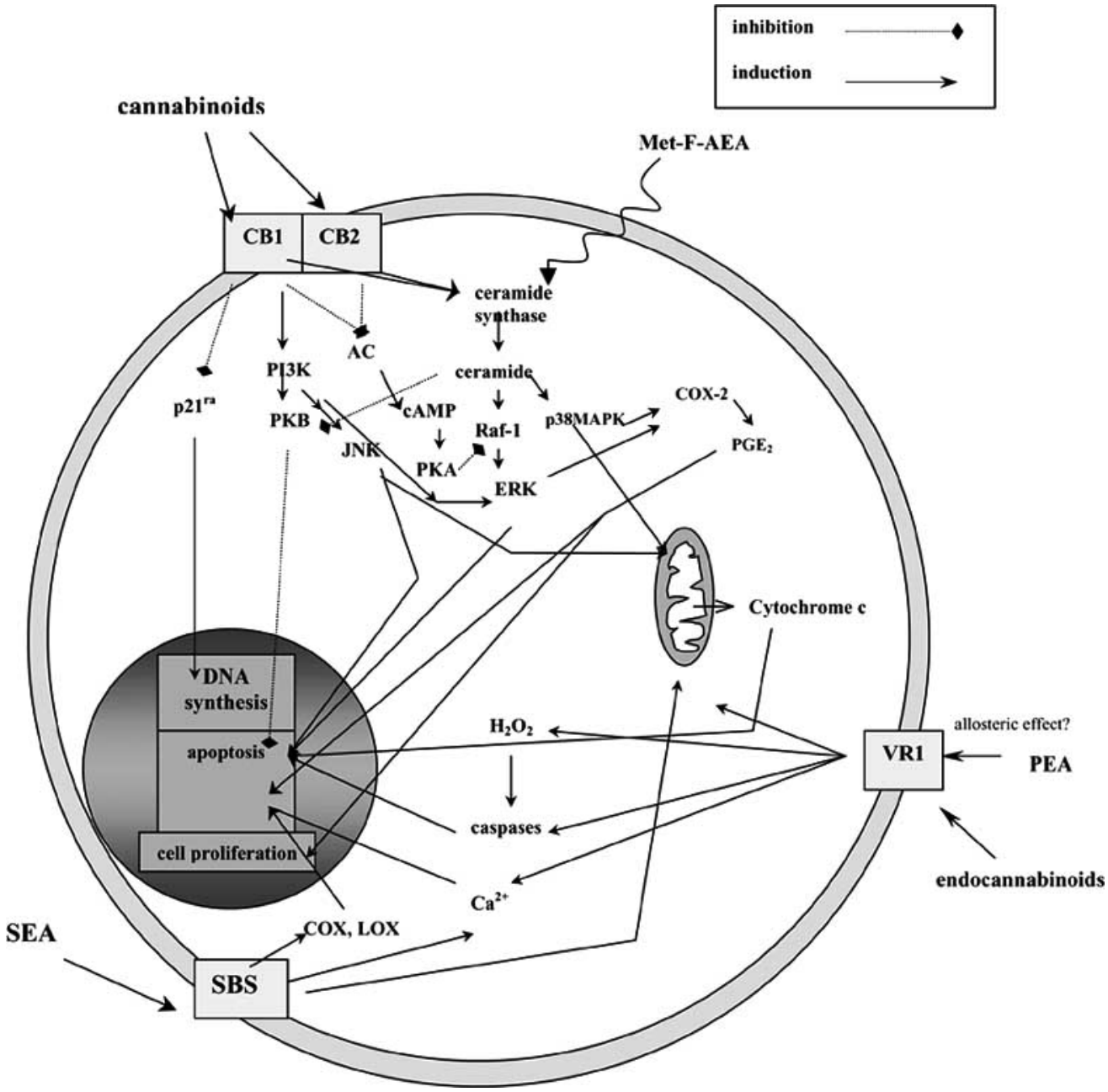

Fig. (1). Cannabinoid mechanisms that involve apoptosis and oxidative stress. Cannabinoids induce *de-novo* synthesis of ceramide which by activating the ERK cascade causes apoptosis *via* both CB1 and CB2 receptors. It can also cause apoptosis through the p38 MAPK pathway. Met-F-AEA up-regulates the synthesis of ceramide not through CB1/CB2 receptors. Another way to cause apoptosis, that is mediated by both cannabinoid receptors, is through the lowering of cAMP synthesis. This leads to the reduction of PKA, which inhibits the ERK cascade, thus preventing apoptosis. ERK can be also activated through PI3K. Sometimes ERK can cause cell proliferation and not apoptosis. CB1 ligands can inhibit p21ras (that induce DNA synthesis), but can also act as anti-apoptotic modulators, *via* PI3K and PKB. VR receptor ligands cause apoptosis by cell content oxidation, mitochondrial uncoupling, intracellular calcium increase and caspase activation. PEA enhances VR ligands' effectivity, maybe by allosteric effect. SEA also causes apoptosis by Ca2+ increase and oxidation by COX and LOX. Methanandamide can also increase COX activity, through non of CB1,CB2 or VR1 receptors, this action is mediated by MAPKs and causes in PGE2 increased levels and increased cancer cell proliferation. In addition CB2 can stop cell proliferation by some unknown mechanism.

In our recent study, a new class of cannabinoid anticancer compounds, cannabinoid quinones, was synthesized. The compounds exerted strong antiproliferative activity on some human cancer cell lines, Jurkat (T cell lymphoma) being the most sensitive. The most effective compound was HU-331 (cannabidiol-hydroxyquinone) [109]. The mechanism of its action is still unclear, but it involves ROS and does not involve cannabinoid receptors, apoptosis and cell cycle alteration (unpublished results).

ANTI-ANGIOGENIC AND ANTI-METASTATIC ACTION OF CANNABINOIDS

The anti-angiogenic properties of cannabinoids have not been thoroughly investigated so far. Leptin (from Greek "leptos" : thin) is known to antagonize cannabinoids in many systems, for example appetite-modulatation and reproduction. As it is a pro-angiogenic factor [65,66], it seems reasonable to expect that cannabinoids may be anti-angiogenic.

Another reason for a possible anti-angiogenic effect by cannabinoids is that they can modulate the response of cells to some growth factors [46-50,86,87,92,93], and presumably, may effect the response to VEGF and FGF, which are the central angiogenic growth factors.

Indeed, numerous cannabinoids (WIN-55,212-2, HU-210, JWH-133 and Δ^9-THC) (Fig. **3**) induce vascular endothelial cell apoptosis, inhibit their migration and inhibit the expression of the pro-angiogenic factors VEGF and Ang2. MMP-2 activity is also blocked through both CB1 and CB2 cannabinoid receptors [67]. In addition, JWH-133-treated tumors showed a pattern of blood vessels characterized by the formation of very small and narrow capillaries, in contrast to untreated tumors where the blood vessels are disorderly heaped on endothelial cells [67]. JWH-133 administration to C6 gliomas bearing mice altered the expression of 10 genes, all of which were directly or indirectly related to VEGF pathway (VEGF-A and B, hypoxia-inducible factor 1-α, angiopoietin-2, Tie-1 and others) [106]. One of the mechanisms by which cannabinoids act as anti-angiogenic factors is by the inhibition of p21ras. As pointed out above, the activation of CB1 receptor blocks p21ras activity, thus inhibiting the growth of KiMol tumors [59]. By inhibiting p21ras, cannabinoids inhibit VEGF transcription (which is induced by p21ras) and induce p57(kip1) kinase (which is inhibited by p21ras) [68]. In addition, Met-F-AEA is able to decrease VEGF-R1 expression [68]. Another study shows that incubation of C6 glioma cells with WIN-55-212,2 inhibited VEGF release into the medium. The spingolipid messenger ceramide was implicated in this attenuation of VEGF production, as pharmacological blockage of ceramide synthesis *de-novo* with fumonisin B1 prevented it [106].

The cannabinoid-induced inhibition of VEGF production was observed also in tumor cells obtained directly from human glioblastoma multiforme biopsies [106]. In contrast to this observation DALN, a cannabinoid agonist, is able to transactivate VEGFR and ERK1/2 phosphorylation through the CB1 receptor [96]. A dramatic inhibitory effect of Met-F-AEA was observed against lung metastatic nodules, induced by the highly metastatic 3LL cells in mice lungs [68]. Another finding that can contribute to the anti-metastatic activity of cannabinoids is their ability to influence cell migration. AEA, DEA (docosatetraenoyl ethanolamide) and HU-210 are capable to inhibit norepinephrine-induced migration of SW480 colon carcinoma cells and MDA-MB-468 breast cancer cells through the CB1 receptor [95].

In skin epithelial tumor cells, one can also see the influence of cannabinoids on a growth factor receptor, EGF-R, which plays a critical role in skin tumor angiogenesis [69]. Cannabinoid administration reduces the levels of EGF-R mRNA and also the degree of its activation (autophosphorylation) [70]. The expression of other pro-angiogenic factors, namely VEGF, PIGF and Ang-2, was also strongly depressed by the treatment with cannabinoids in skin tumors [70].

SOME PRO-CANCER EFFECTS?

Regardless of the anticancer activity of cannabinoids, sometimes they can act also as pro-cancer agents, especially in low concentrations, acting mostly through growth factors and their receptors activation/induction.

Treatment of some cancer cell lines (NCI-H-292 lung carcinoma, 5637 bladder carcinoma, U373-MG glioblastoma, 1321N1 astrocytoma and A498 kidney carcinoma) with THC, AEA, HU-210 or WIN55,212-2 leads to rapid cleavage of proAmphiregulin (proAR) and/or proHeparin-binding EGF-like growth factor (proHbEGF) at the cell surface by tumor necrosis factor α converting enzyme (TACE/ADAM17). ProAR and proHbEGF cause EGFR tyrosine phosphorylation and downstream activation of PKB/Akt and ERK1/2 pathways, both of which mediate cell proliferation/survival [93]. However, in larger concentrations, THC causes cell death [93]. This biphasic effect of cannabinoids has previously been noted with numerous other cannabinoid effects [103-105].

As mentioned above, cannabinoids transactivate the PDGF receptor in C6 glioma cells [38]. In N18TG2 neuroblastoma cells VEGFR transactivation was caused by the cannabinoid agonist DALN through the CB1 receptor, and it also led to ERK 1/2 phosphorylation [96]. In LCNaP prostate cancer cells, the androgen receptor is upregulated by Met-F-AEA through CB1 and CB2 receptors with the involvement of PI3K [86], methanandamide up-regulates the androgen receptor in these cells and is even able to enhance the proliferation in these cells. However, the proliferation enhancement is seen only in cells treated with very low (0.1μM), methanandamide concentrations; in the concentrations above 1μM methanandamide causes cell death [87]. In another prostate cancer cell line, PC3, low concentrations of THC or Met-AEA enhance their proliferation through PKB/Akt activation and NGF induction. The latter is mediated by the activation of ERK1/2 by Raf-1 that itself is activated by PI3K, through both cannabinoid receptors [92].

Met-AEA administration to 3LL (Lewis lung carcinoma) and L1C2 (alveolar cell carcinoma) lines resulted in an increased rate of tumor growth, which was not prevented by CB1 and CB2 antagonists. Met-AEA increased PGE2 levels at the tumor site, probably through MAPKs and COX pathway, as p38 MAPK, ERK and COX-2 inhibitors abrogated the induction of PGE2 production, a COX-2 inhibitor also blocked Met-AEA-induced tumor growth in-vivo [97]. The mechanism of MAPKs' activation in these cells is unknown. In another cell line, H4 human neuroglioma cells, Met-AEA induced the same pathways: MAPKs‡COX-2‡PGE2 through enhancement of *de-novo* ceramide synthesis. This effect is not mediated by CB1, CB2 and VR1 receptors [98], but is probably mediated by membrane lipid rafts, as MCD, a membrane cholesterol

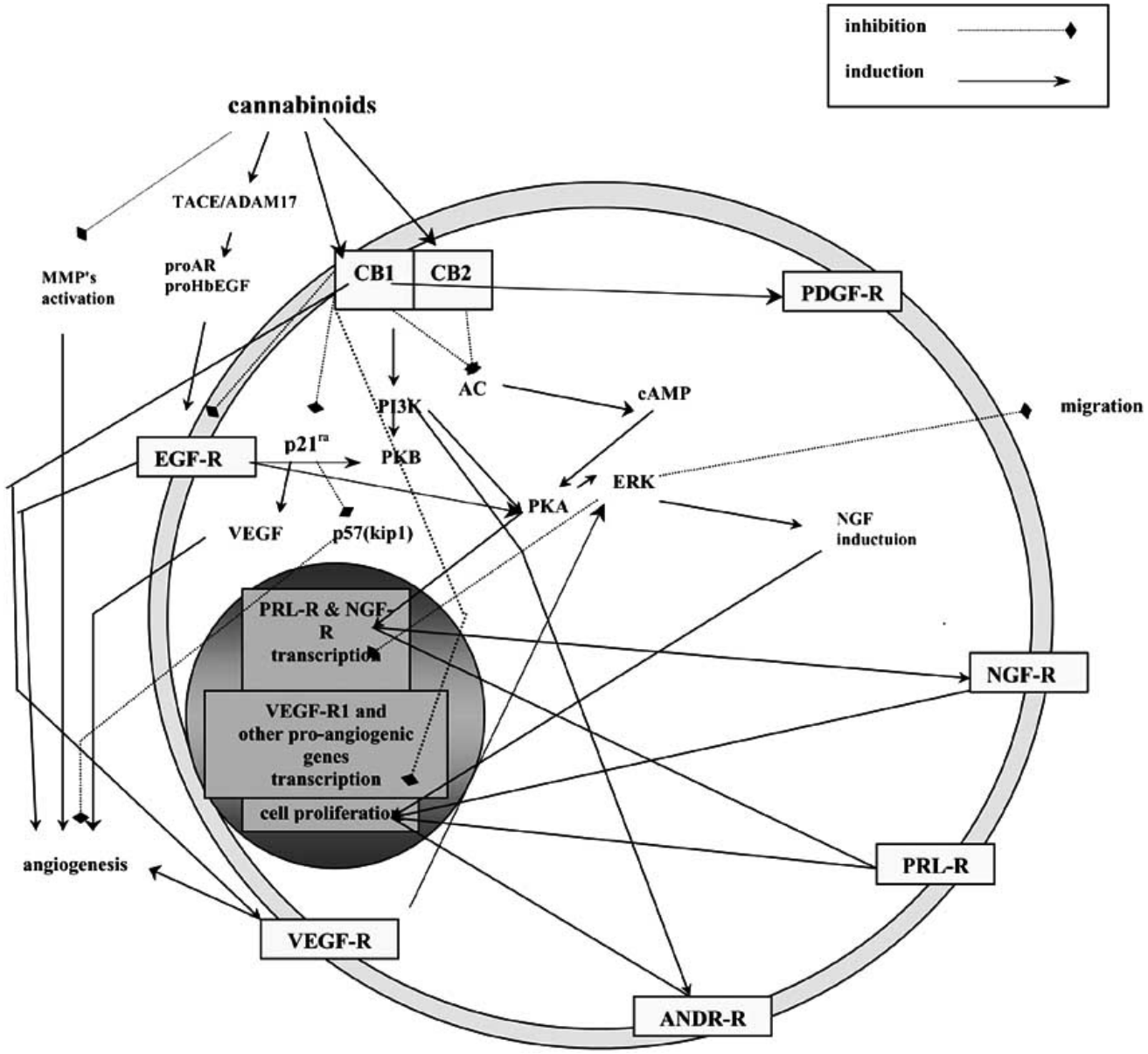

Fig. (2). Cannabinoid mechanisms that involve growth factors modulation and angiogenesis. Cannabinoids reduce PKA levels through AC and activate ERK. PKA induces (and ERK reduces) *trk* and PRL-R transcription, which is especially important in growth factor-dependent cancer cell lines, like breast and prostate cancer. ERK may be activated by growth factor receptors, like EGF-R or VEGF-R. Cannabinoids (in different concentrations and cell lines) are capable of both down-regulating and up-regulating these receptors, thus influencing ERK activation and cell proliferation. Cannabinoids inhibit p21ras, which induces VEGF transcription and inhibits p57(kip1) - an anti-angiogenic protein; hence CB1 is anti-angiogenic. Cannabinoids can activate VEGF receptor, one of the main angiogenic receptors. CB1 can transactivate PDGF-R and inhibit EGF-R and also VEGF-R, but cannabinoids are able also to activate EGF-R and up-regulate the androgen receptor.

depletor, inhibits R(+)-Met-AEA-induced COX expression and subsequent formation of PGE2 [113]. However, in this case, apoptosis rather than proliferation is observed [115].

In a recent study, a novel mechanism of proliferation by endocannabinoids was discovered. In a NIH3T3 SPIα cell line (cells overexpressing phosphatidyl inositol transfer protein α), which shows an enhanced PLA2-mediated degradation of PI and high amounts of arachidonic acid, the rate of proliferation was significantly increased. Conditioned medium (CM) from these cells increases the proliferation of wtNIH3T3 cells, suggesting the production of some mitogenic factor by SPIα cells. Apparently this mitogenic factor is a COX-2-dependent unidentified eicosanoid (as COX-2 inhibitor blocked the proliferative activity of SPIα cells CM). It may be an endogenous cannabinoid factor, as SR141716A, a synthetic CB1 receptor antagonist, reduced its activity [99].

It is hard to find any correlation between cannabinoid action through the known CB receptors and their proliferative/antiproliferative effects. As it is seen in this

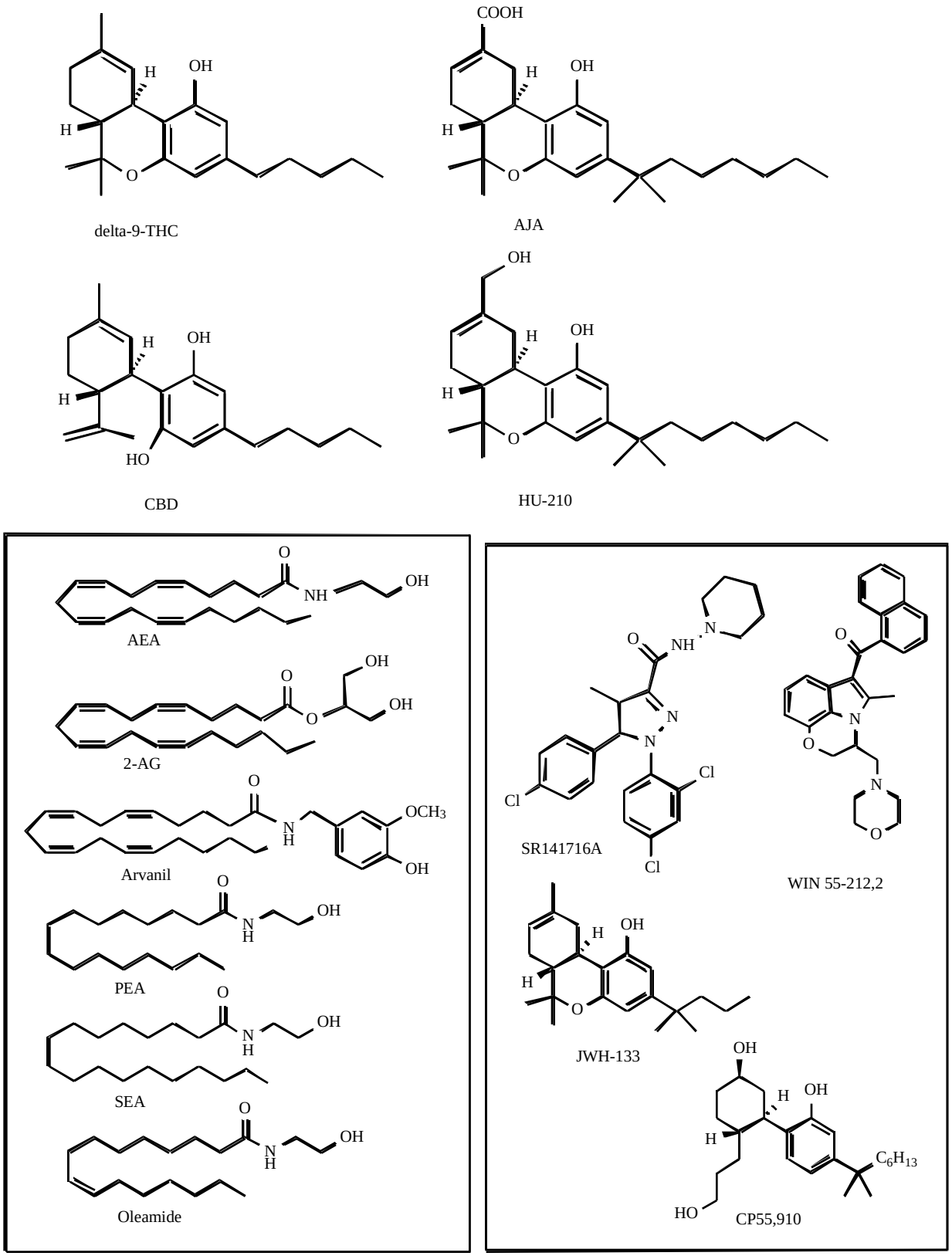

Fig. (3). A. Plant cannabinoids, B. Classical synthetic cannabinoids, C. Endocannabinoids D. Other synthetic cannabinoids.

chapter, the proliferative action of cannabinoids can be CB-receptors-mediated [87,96,99]; cannabinoid receptors are also able to protect the cells from VR1-mediated death [53,94,112], however in other cases, the growth induction is not mediated by CB1/CB2/VR1 receptors [97].

CONCLUDING REMARKS

The role of endocannabinoid system in the regulation of physiological processes is complicated. One of their actions is the control of cell fate/survival (see ref.71-74 for reviews).

Among the cells whose fate is regulated by cannabinoids are also cancer cells. Cannabinoids can interfere with the action of some growth factors and thus block growth factor-dependent cancers [46-48,75,86] and also decrease new blood vessel spreading into the cancer [67,68,70]. The mechanisms of the regulation of cell fate by cannabinoids differ between different cell lines and depend on the concentration of the cannabinoids. Oxidative stress is involved in many cases of cannabinoid-induced cancer cell death [35,36,53,58,91,115]. In glioma cells, cannabinoids act preferentially through the ceramide pathway and apoptotic death [23,25,30,31,98,115], but there are other mechanisms, which include oxidative stress damage [35,36,91]. In some cases, cannabinoids act through both cannabinoid receptors [25,31] (or only through CB2 [31,37,91]), while in other cases, both cannabinoid receptors and vanilloid receptors are involved in cell death [35,90], or even by action on vanilloid receptors alone, when the role of cannabinoid receptors may actually be protective [53,94,112]. However, CB receptors might not be really proliferative/protective, maybe they are just the targets that bind cannabinoids, but are not involved in any action in these cases. Thus, CB antagonists actually synergize with cannabinoids (that act through VR1) just by displacing them from inactive targets.

The effect on growth factor-dependent (breast and prostate) cell lines is quite different. Here, cannabinoids mostly kill the cells by blocking the cell cycle [46,86] and downregulating the growth factors (or their receptors) needed for the proliferation [46-48,86] of these cells. These effects are mostly mediated by CB1 receptor [46-48,86], but in some cases, both cannabinoid receptors [87] or vanilloid receptors [48] are involved.

In some cases, however, cannabinoids are able to upregulate/transactivate some growth factor receptors [38,87,92,93,96]. It happens mostly in low concentrations of cannabinoids/low exposal times. Here again, there is no dependence on cannabinoid/vanilloid receptors. In some cases, both CB receptors are involved [86,87,92], in other cases- only CB1 [96,99]. A biphasic dose profile of cannabinoid action was suggested many years ago [101,102] under various experimental conditions [103-105] and may be of importance in their actions on cancer cells. Endocannabinoids are known for their stimulatory/ suppressing effects on the levels of some hormones [42,43] and suppress the regulatory action of the hypothalamo-pituitary-adrenal axis [44,45]. The above discussion indicates that they play an important role in the regulation of many other growth-factors.

The data summarized in this review strongly indicate that the endocannabinoid system is an important control system, which, under certain circumstances, may actually lead to cancer formation/enhancement. This assumption is supported by the fact that endocannabinoid concentration is six-fold smaller in some cancers, like meningioma and glioblastoma, than in the surrounding healthy tissue [76]. In contrast, malignant glioma cells express more CB2 receptors than healthy cells [31]. This upregulation of CB receptors, or increased cannabinoid concentrations, has been also noted in some neurodegenerative diseases or in neurotraumatic pathology [110,111]. This phenomenon, noted in different disease states, suggests that the cannabinoid system may represent a general protective entity. The picture is not straightforward as KiMol (transformed thyroid) cells exhibited more CB1 receptors when treated by Met-F-AEA, while non-transformed FTRL-5 cells exhibited fewer receptors than non-treated cells [59]. In this manner, cannabinoids can inhibit the growth of cancer thyroid cells much more effectively in transformed than in non-transformed cells. Another system where cannabinoids are able to distinguish between less/more malignant cells is metastasis. Indeed, anandamide inhibits the growth of metastasis-derived thyroid cells more efficiently than that of primary thyroid carcinoma derived cells [68]. Of further interest is the possibility that the endocannabinoid system represents one of the defense mechanisms of the body against cancer proliferation. The treatment of cancer cells with VDM11 and AA-5-HT which inhibit anandamide transport into the cell and anandamide hydrolysis respectively, potentiate anandamide-mediated inhibition of C6 glioma cell proliferation [85]. They are also able to inhibit KiMol tumor growth *in-vivo*, by enhancing intratumoral endocannabinoids levels [114]. These compounds have a high potential in anticancer therapy.

It now seems that there is no clear structure-activity relationship in cannabinoid anticancer effects (see Fig. **3** for cannabinoids' structures). Plant cannabinoids (such as THC, CBD and synthetic compounds that resemble plant cannabinoids such as HU-210, AJA), endocannabinoids (such as AEA, 2-AG and the synthetic stable AEA's analog Met-AEA) and synthetic cannabinoids (such as WIN-55-212,2, JWH-133) are all able to act through both CB1 and CB2, or through other mechanisms as anti-cancer/pro-cancer agents. The results strongly depend on cell type and on the concentration of the compounds. Endocannabinoids have an advantage of action through VR1 receptors, but even here the picture remains unclear. The action through a receptor is usually assayed by adding a receptor antagonist and observing its interference with the effect of the agonist. In studies with endocannabinoids, capsazepine is usually used as VR1 antagonist, but it is not very selective. When SB366791, a more selective VR1 antagonist was used, it was less effective than capsazepin in preventing AEA-mediated C6 glioma cell death, suggesting that some VR1-independent mechanism is involved [90]. This can also explain the anticancer action of some compounds like oleamide [49], PEA [50,52], SEA [36],cannabidiol [91] and cannabigerol [62], all of which have minimal affinity to cannabinoid/vanilloid receptors. It can also explain some "non-receptor-mediated" anticancer effects[61,115] of the compounds that are able to bind these receptors in other cases. A new target for cannabinoid action (CB3?) was found recently [116,117]. Additional cannabinoid receptors may exist. Without this information, it seems almost impossible to determine the structure-activity relationship for the cannabinoid anticancer effects.

We would like to allude to the anti-emetic and appetite inducing properties of cannabinoids, as well as their pain-reducing properties. Anorexia and cachexia are diagnosed in more than two-thirds of all cancer patients with advanced disease, and are independent risk factors for morbidity and mortality. Anorexia, nausea and vomiting often are described as more significant inhibiting factors for quality of life of cancer patients than even intense pain. THC is licensed as an

anti-emetic drug in cancer patients receiving chemotherapy, and has shown significant stimulation of appetite and increase of body weight in cancer patients [77]. Cachexia occurs secondarily as a result of a functional inability to ingest or use nutrients. This can be related to mechanical interference in the gastrointestinal tract, such as obstruction or malabsorption, surgical interventions, or treatment-related toxicity. And in patients receiving chemotherapy or radiation therapy, nausea, vomiting, taste changes, stomatitis and diarrhea can all contribute to weight loss [78,79]. Cannabinoids (especially THC) can reduce cancer cachexia [80] as well as cancer pain [81,82], thus providing useful therapy, both against the side effects of the anticancer drugs used today and possibly as anticancer drugs in their own right.

In summary, cannabinoids possess some anticancer activity. Possibly they may represent a new class of anticancer drugs that retard cancer growth, inhibit angiogenesis and the metastatic spreading of cancer cells.

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ABBREVIATIONS

AC	=	Adenylate cyclase
2-AG	=	2-arachidonoyl glycerol
AJA	=	Ajulemic acid
AEA	=	Arachidonoyl ethanolamide, anandamide
cAMP	=	Cyclic AMP
ASK-1	=	Apoptosis signal regulating kinase 1
Ang-2	=	Angiopoietin-2
CB1/CB2	=	Cannabinoid receptors 1/2
CBD	=	Cannabidiol
COX	=	Cyclooxygenase
DEA	=	Docosotetraenoyl ethanolamide
DALN	=	Desacetyllevonantradol
EGF-R	=	Epithelial growth factor receptor
ERK	=	Extracellular regulated kinase
FAAH	=	Fatty acid amide hydrolase
FGF	=	Fibroblast growth factor
IL-2	=	Interleukin-2
JNK	=	C-jun N-terminal kinase
LOX	=	Lypooxygenase
MAPK	=	Mitogen activated protein kinase
MCD	=	Methyl-beta-cyclodextrin
Met-AEA	=	Methanandamide
Met-F-AEA	=	Methfluoroanandamide
MMP-2	=	Matrix methalloproteinase-2
NGF-R	=	Nerve growth factor receptor
PDGF-R	=	Platelet derived growth factor receptor
PEA	=	Palmitoyl ethanolamide
PGE2	=	Prostaglandin E2
PI3K	=	Phosphatidyl inositol 3 kinase
PKA	=	Protein kinase A
PKB	=	Protein kinase B
PlGF	=	Placental growth factor
PRL	=	R prolactin receptor
proAR	=	Proamphiregulin
proHbEGF	=	Pro-heparin-binding EGF-like growth factor
ROS	=	Reactive oxygen species
SBS	=	SEA binding site
SEA	=	Stearoyl ethanolamide
TACE	=	Tumor necrosis factor α converting enzyme
TG	=	Triglyceride
THC	=	Tetrahydrocannabinol
VEGF-R	=	Vascular endothelial growth factor receptor
VR	=	Vanilloid receptors

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