

dependent on the activation of CB1 receptors, although receptor-independent cell death has also been described (Sanchez et al., 1998). In some cases, CBD actually enhanced primary tumor growth *in vivo*, as indicated by increased thymidine incorporation (Carrier et al., 2006). Such results suggest an indirect anti-inflammatory effect of CBD, as it could improve the ability of tumors to escape immune surveillance. On the other hand, CBD has also been shown to exert antitumor actions in some glioma models (Torres et al., 2011). Whether CB receptor stimulation triggers cell death/apoptosis or proliferation/survival could, probably, be determined by different signaling coupling between cannabinoids and tumor/transformed cells or normal cells.

Recent observations indicated that Δ^9 -THC and a CB2 receptor agonist (JWH-015) can induce autophagy and cell death in various tumor cells, including glioma, pancreatic and hepatic cancer cells, while they do not affect this process in non-transformed cells (Salazar et al., 2009; Vara et al., 2011). Pharmacological or genetically elicited inhibition of autophagy prevented cannabinoid-induced cell death and apoptosis; blocking apoptosis alone prevented cell death but not the autophagy induced by these compounds. It was thus suggested that autophagy induction is part of the mechanism by which cannabinoids promote apoptotic death in cancer cells/tumors (Salazar et al., 2009; Vara et al., 2011).

Cell cycle arrest involves the up-regulation of the p53 protein which will differentially alter pro- and anti-apoptotic protein levels (i.e. increase of the pro-apoptotic protein Bax levels and lower those of the anti-apoptotic protein Bcl2, respectively, thereby shifting the ratio towards Bax) which ultimately leads to activation of caspases that play an essential role in triggering apoptosis (Sarfaraz et al., 2006). Activation of either CB1 or CB2 receptors also inhibits AC activity and lowers both cyclic adenosine monophosphate (cAMP) levels and protein kinase A (PKA) activity, thereby causing down-regulation of gene transcription, leading to apoptosis (Guzmán, 2003; Kogan, 2005; Bifulco et al., 2008; Sarfaraz et al., 2008). Activation of TRPV1 receptors also leads to increases in intracellular levels of both hydrogen peroxide (H₂O₂) and/or calcium or the release of cytochrome C from mitochondria, causing apoptosis through both distinct and overlapping mechanisms (Maccarrone et al., 2000).

AEA inhibits cell proliferation through CB1, but not CB2 receptors (Melck et al., 2000; Mimeault et al., 2003), whereas induction of apoptosis involves both CB1 and CB2 receptors, as shown in prostate cancer cells (Mimeault et al., 2003). Inhibition of endocannabinoid hydrolysis by methyl arachidonyl fluorophosphonate (Nithipatikom et al., 2004), which targets fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), and by CAY10401 (Endsley et al., 2008), which targets FAAH, suggests that elevation of endocannabinoids inhibits cell proliferation. Inhibition of 2-AG hydrolysis by 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP), a compound containing a trifluoromethylketone moiety, has also been shown to inhibit prostate cancer cell proliferation (Nithipatikom et al., 2005; Endsley et al., 2007) through a CB1-dependent mechanism (Nithipatikom et al., 2005). Synthetic endocannabinoids have also been shown to exhibit anti-proliferative properties (Galve-Roperh et al., 2000; Sanchez et al., 2001; Duntsch et al., 2006)

CB agonists reduce neoangiogenic growth in different malignant cells by inhibiting the EGF (Casanova et al., 2003; Sarfaraz et al., 2006). Moreover, neurotrophins are also involved in VEGF mediated pathways. For instance, CB agonists stimulate the synthesis of brain-derived neurotrophic factor (BDNF) and this is followed by a protection of neurons against kainic acid excitotoxicity (Khaspekov et al., 2004). Recently, an increased CB1 receptor protein expression was observed in combination with an up-regulation of BDNF in cases of cerebral lesions (De March et al., 2008). Also, the nerve growth factor (NGF) has been suggested as a potential transmitter for tumor neoangiogenesis by its interaction with VEGF. NGF increases VEGF mRNA expression and the accumulation of VEGF protein in pheochromocytoma cells (Middeke et al., 2002).

BRAIN TUMORS

Cell constituents of healthy brain include neurons and glia, with glia further classified as astrocytes, oligodendrocytes, or microglia. The term glioma broadly classifies primary neoplastic tissue of glial origin, with astrocytomas representing the most common group.

Expression of receptors and correlation with clinical parameters

Gliomas, the most common primary brain tumors, account for more than 40% of all CNS neoplasms and are highly resistant to available therapeutic approaches, including radiation and chemotherapy (Hosli et al., 1998; Galanis and Buckner., 2000). Despite recent progress in characterizing the molecular pathogenesis of gliomas and current efforts to develop more effective treatment strategies, these tumors have a generally poor prognosis, with median survival time for patients with advanced tumors (grade III/IV) being approximately 1 year (Ohgaki and Kleihues, 2005; Burnet et al., 2007).

Astrocytomas, a subtype of gliomas, are highly aggressive tumors that express glial fibrillary acidic protein (GFAP) and have fibrillary cytoplasm and angular nuclei (Fomchenko et al., 2006). The tumor mass often contains a high degree of cellular heterogeneity and displays significant microvascular proliferation. The World Health Organization classifies astrocytomas into low (I and II) or high grade (III and IV), depending on their particular location and growth rate. Anaplastic astrocytomas (grade III) and malignant GBM (grade IV) represent the most aggressive primary tumors of the CNS and account for nearly one third of all diagnosed brain tumors (Kleihues et al., 2002). Their precise cellular origin remains debated principally because of their heterogeneity and broad etiology. Possible origins include terminally differentiated astrocytes, glial precursors or cancer stem cells (Fomchenko and Holland., 2006; Fomchenko and Holland., 2006).

GBM ranks among the most malignant and frequent primary brain tumor in adults. Median survival rate with optimal treatment is lower than 15 months (Laperriere et al., 2002). The incidence of GBM is 5 per 100,000, with men more frequently affected than women. GBM is characterized by fast growth and a particular dependence on blood vessel formation for survival.

CB1 receptor

By quantitative reverse transcription polymerase chain reaction (RT-PCR), western blot and confocal immunohistochemistry, CB1 has been shown to be the most important cannabinoid receptor subtype in human astrocytoma tissue and in glioma cells. In glioma tissue CB1 was mainly detected on astroglial cells/GFAP-positive glioma cells, although not frequently on proliferating malignant cells (Held-Feindt et al., 2006).

CB1 receptor expression levels in astrocytoma and glioma tissues and tumor-associated endothelial cells were not significantly higher than in normal brain tissues, being unrelated to the grade of malignancy (Sanchez et al., 2001; Schley et al., 2009). However, significant differences in CB1 receptor expression between malignant and control tissues were noted, which reached statistical significance only in glioblastoma samples (CB1 receptor immunoreactivity was significantly lower in GBM group than in control group - Dunnett's test $p < 0.05$) (De Jesus et al., 2010).

CB2 receptor

CB2 receptor, expressed in both macrophages and brain microglia, has been implicated in the control of fundamental neural cell processes, such as proliferation, differentiation and survival (Fernandez-Ruiz et al., 2007; Benito et al., 2008). Moreover, an inverse relation between CB2

receptor expression and the grade of cell differentiation is evident in neurones and neuroglial cells (Palazuelos et al., 2006), suggesting that CB2 receptor might function as a “cell de-differentiation signal” by favouring a non-differentiated, proliferative state (Fernandez-Ruiz et al., 2007). In fact, CB2 receptors are normally expressed at very low levels in non-pathologic human brain but their expression significantly increases in microglial elements and astrocytes after pathological neuroinflammatory insults (Fernandez-Ruiz et al., 2007; Benito et al., 2008). Thus, brain CB2 receptors are up-regulated in Alzheimer’s and Huntington’s diseases, encephalitis and multiple sclerosis (Benito et al., 2003; Fernandez-Ruiz et al., 2007; Benito et al., 2008).

Human astrocytic tumors, both benign (eg. pilomyxoid astrocytoma, juvenile pilocytic astrocytoma, subependymal giant cell astrocytoma-SEGA) and malignant (eg. GBM, giant cell glioblastomas) exhibit significant levels of CB2 expression, due to the main microglial location of these receptors. In anaplastic astrocytomas and GBM, microglia may constitute up to 30% of all tumor cells (Badie and Schartner, 2001). The extent of CB2 expression correlated with the tumor histopathological grade, as it also appears in other tumors, such as breast cancer (Caffarel et al., 2006). Furthermore, CB2 receptor expression was higher in astrocytomas/glioblastomas than in oligodendrogliomas, ependymomas or meningiomas of the same grade (Ellert-Miklaszewska et al., 2007). Expression levels of CB2 receptors in human GBM were considerably increased when compared to CB1 receptor expression (Sanchez et al., 2001; Schley et al., 2009). Whether the increased CB2 receptor expression found in human gliomas derives directly from them or is due to invading immune or endothelial cells remains unclear (Cudaback and Stella, 2007). In this way, it has been speculated that microglial/macrophage infiltration in human astrocytomas is responsible for the main part of CB2 receptor expression in solid gliomas (Held-Feindt et al., 2006). Moreover, enhanced CB receptor staining was identified on endothelial cells of vessels adjacent to GBM, suggesting that CB2 receptors on tumor cells may be crucial for GBM growth by their interaction with endothelial cells. The importance of VEGF signalling for neoangiogenesis of GBM and its regulation by selective CB2 agonists and neurotrophic factors represents an intriguing therapeutic target for GBM treatment in humans (De Jesus et al., 2010).

TRPV receptors

Gliomas, both *in vitro* and *ex vivo*, express not only CB1 and CB2, but also TRPV1. Furthermore, in contrast with results obtained with Δ^9 -THC (Galve-Roperh et al., 2000; Sanchez et al., 2001), AEA-induced death of glioma cells was not mediated via CB1/CB2, but through TRPV1 (Contassot et al., 2004).

TRPV2 receptor was also significantly expressed (mRNA, protein) in benign astrocyte tissue but decreased progressively in glioma tissue with increasing tumor histological grading (Nabissi et al., 2010).

Effects of cannabinoids on cell proliferation and angiogenesis

***In vivo* evidence**

During the last years, several studies have demonstrated that cannabinoids induce apoptosis of glioma cells *in vitro* (Sanchez et al., 1998) and inhibit angiogenesis of gliomas *in vivo* (Velasco et al., 2007). Studies using animal models demonstrated that local administration of Δ^9 -THC or the synthetic cannabinoid WIN-55,212-2 (a mixed CB1/CB2 agonist) reduced the size of tumors generated by intracranial inoculation of C6 glioma cells in rats, without affecting healthy brain tissue. This led to complete glioma eradication and prolonged survival in one third of the cannabinoids-treated rats (Galve-Roperh et al., 2000). Studies performed in mouse xenograft models with intratumoral and intraperitoneal drug administration demonstrated that non-

psychoactive phytocannabinoid CBD (Massi et al., 2004), Δ^9 -THC, WIN-55,212-2, JWH133(a CB2-selective agonist) (Sanchez et al., 2001) or the novel synthetic cannabinoid KM-233 (Duntsch et al., 2006) blocked the proliferation of tumors derived from the rat C6 glioma cell line and also from GBM cells obtained from human tumors (Galve-Roperh et al., 2000; Sanchez et al., 2001) implanted subcutaneously in the flank of immune-deficient mice.

The best characterized mechanism of cannabinoid-induced (Δ^9 -THC and WIN-55,212-2) cell death of glioma cells involves sustained accumulation of pro-apoptotic sphingolipid ceramide, which modulates signaling pathways crucial in the control of tumor cell growth and survival, like ERK1/2 activation (Galve-Roperh et al., 2000; Sanchez et al., 2001; Carracedo et al., 2006). It was also reported that cannabinoids down-regulated PI3K, Akt and ERK signaling pathways, and activated proapoptotic function of Bad protein, leading to apoptosis induction (Ellert-Miklaszewska et al., 2005). Selective CB2 receptor agonists, such as JWH133, are supposed to stimulate only the ceramide synthesis process, which is sufficient to turn on the cell death program (Gomez del Pulgar et al., 2002; Sanchez et al., 2001; Sarfaraz et al., 2006).

A great deal of attention is currently focused on abnormal cell cycle regulation (Rich and Bigner, 2004), for example, dysfunction of the p53 pathway (p14ARF, HDM2, and p53) and the RB1 pathway (p15INK4B, p16INK4A, CDK4, Cyclin D1 and RB1). Mitogenic signaling pathways may also be dysfunctional, for example, RAS, MAPK, and PI3K coupled to growth factor receptors (IGF1R, EGFR, and PDGFR) (Fomchenko and Holland, 2006).

Several studies showed that cannabinoids could exert their anti-proliferative effects by inducing ROS-dependent cell death. It was also demonstrated that the anti-proliferative effects of AEA and 2-AG on glioma cells could be totally inhibited by the antioxidant α -tocopherol (Jacobsson et al., 2001). The antiproliferative, apoptotic effects of cannabinoids appear to be tumor-selective, affecting tumor cells but not normal brain cells such as astrocytes, oligodendrocytes and neurons; indeed the latter appeared to be protected by the cannabinoid treatment (Galve-Roperh et al., 2000; Gomez Del Pulgar et al., 2002).

Δ^9 -THC administration to mice with human astrocytoma resulted in increased TRB3 expression, inhibition of mTOR signaling pathway, appearance of autophagy markers and caspase-3 activation. Such findings indicate that cannabinoid promotes the autophagy-mediated cell death through stimulation of ER stress in human glioma cells (Salazar et al., 2009).

Spontaneous regression in brain tumors has been previously described in non-neurofibromatosis type-1 (NF1)-associated pilocytic astrocytomas of the cerebellum (Steinbok, 1994; Palma et al., 2004; Gunny et al., 2005; Saunders et al., 2005; Steinbok et al., 2006), thalamus (Balkhoyor and Bernstein, 2000), temporal lobe (Rozen et al., 2008) and tumors associated with NF1, but not in the rare fornix/septum pellucidum location. However, spontaneous regression of septum pellucidum/forniceal pilocytic astrocytomas in the absence of NF-1 has been reported in two children who underwent craniotomy and subtotal excision, but did not receive any conventional adjuvant treatment. The tumors regressed over the same period of time that cannabis was consumed via inhalation, raising the possibility that the cannabis played a role in tumor regression (Foroughi et al., 2011).

Since stereotaxic injection of chemotherapeutic compounds directly into human brain tumor masses constitutes a routine approach for neurosurgeons, high concentrations of cannabinoids can easily be delivered by this technique (Guzman et al., 2006). There are two additional advantages to delivering high concentrations of cannabinoids directly into the tumor mass. Malignant transformation of astrocytomas is associated with an increase in CB receptor expression (Sanchez et al., 2001; Held-Feindt et al., 2006) and this increase in expression precludes the use of low cannabinoid concentrations known to activate these receptors. Conversely, local injection of high cannabinoid concentrations will induce apoptosis in all astrocytoma subclones (independently of CB1 and CB2 receptor expression), which constitutes an asset when considering the phenotypic heterogeneity that astrocytomas adopt during malignant transformation (Wen and Kesari, 2008; Hambardzumyan et al., 2008). Thus, high cannabinoid concentrations constitute the preferred

regimen for neurosurgeons and neurooncologists to use when treating malignant astrocytomas with this class of compounds (Cudaback et al. 2010).

***In vitro* evidence**

Normal tissue toxicity limits the efficacy of current treatment modalities for GBM. WIN 55,212-2 and Δ^9 -THC significantly reduced *in vitro* the growth of various human GBM cell lines examined, including SF126, U87MG, U251, U373MG, and SF188 (McAllister et al.; 2005). Δ^9 -THC decreases cell proliferation and increases cell death of human GBM cells more rapidly than WIN 55,212-2 (McAllister et al.; 2005). The effects of Δ^9 -THC and WIN 55,212-2 on GBM cells were partially the result of CB receptor activation. The observed potencies were surprising because the synthetic aminoalkylindole WIN 55,212-2 has a significantly higher affinity for both CB1 and CB2 receptors compared to the classical cannabinoid Δ^9 -THC (Showalter et al.; 1996). Similarly, WIN55,212-2 is consistently more potent and efficacious compared to Δ^9 -THC when CB receptor pathways are evaluated (Felder and Mitchell, 1995), including effects on cAMP, MAPK, and ion channel activity (Pertwee, 1997). It has been shown that CB receptor-mediated generation of ceramide, leading to long-term stimulation of ERK, plays a pivotal role in inducing GBM cell death (Guzman, 2003). The same concentration of Δ^9 -THC that significantly inhibited proliferation and increased human GBM cells death had no significant impact on human primary glial cultures. Evidence of selective efficacy with WIN 55,212-2 was also observed but the selectivity was less profound, and the synthetic agonist produced a greater disruption of normal cell morphology compared to Δ^9 -THC (McAllister et al.; 2005). Cannabinoids have been shown to induce apoptosis only in astrocytoma cells expressing low CB receptor levels that couple to ERK1/2. In contrast, cannabinoids do not induce apoptosis in astrocytoma cells expressing high receptor levels because these now also couple to the prosurvival signal Akt. Remarkably, cannabinoids applied at high concentration induce apoptosis in all subclones independently of CB1, CB2 and Akt, but still through a mechanism involving ERK1/2 (Cudaback et al., 2010).

After showing that C6 sensitivity to the chemotherapeutic agent tamoxifen is increased by coadministration of Δ^9 -THC (Jacobsson et al., 2000), this group found that several other cannabinoids, including AEA, 2-AG, CP 55,940, and the CB2 agonist, JWH-015, also inhibited C6 proliferation (Jacobsson et al., 2001; Fowler et al., 2003). Several other cannabinoids have also been shown to induce apoptosis in C6 cells, including the endocannabinoid analogue stearoylethanolamide (Maccarrone et al., 2002; Ellert-Miklaszewska et al., 2005). Furthermore, ajulemic acid, a non-psychotropic synthetic Δ^9 -THC analogue, is more effective than Δ^9 -THC at inhibiting C6 and U87 (a human glioma line) proliferation in culture (Recht et al., 2001). While the anti-proliferative effect of endocannabinoids is blocked by CB receptor antagonists, the anti-proliferative effect of synthetic agonists was not (Jacobsson et al., 2001). Interestingly, AEA also induced apoptosis in a variety of human glioma cell lines through TRPV1 (Maccarrone et al., 2000; Jacobsson et al., 2001; Jonsson et al., 2003; Contassot et al., 2004), and chemical disruption of lipid rafts blocked this effect (Bari et al., 2005). In rat C6 glioma cells, the high affinity glycine transporter GLYT1 has been shown to be attenuated via protein kinase C- α (PKC- α) pathways (Morioka et al., 2008). GLYT1 is crucially involved in the clearance of the inhibitory neurotransmitter glycine from synapses, and thus its downregulation enhances the efficacy of glycine-mediated inhibition. The finding that CB-mediated effects are impaired by PKC-inhibitors in neuroblastoma cells (Rubovitch et al., 2004) indicates that the recruitment of CB receptors may involve downstream PKC- α and GLYT1 regulation (Guzman et al., 2006; Blazquez et al., 2008) and thereby attenuate glioblastoma growth and proliferation.

A novel cannabinoid chemotherapeutic, KM-233, is a classical cannabinoid with good blood brain barrier penetration that possesses a selective affinity for CB2 receptors relative to Δ^9 -THC. KM-233 was as efficacious in its cytotoxicity against human U87 glioma as Δ^9 -THC, and superior to the commonly used anti-glioma chemotherapeutic agent, bis-chloroethylnitrosourea (BCNU). An advantage of the combined administration of KM-233 and BCNU is that the former works through

CB1/CB2 receptor activation and the latter via DNA alkylation and it is therefore possible that considerable synergy may be seen. The cytotoxic effects of KM-233 against human glioma cells *in vitro* occur as early as two hours after administration, and dosing of KM-233 can be cycled without compromising cytotoxic efficacy and while improving safety. Cyclical dosing of KM-233 to treat U87 glioma in a severe combined immunodeficiency (SCID) mouse xenograft side pocket model was effective at reducing the tumor burden with both systemic and intratumoral administration (Duntsch et al., 2006). *In vitro* studies identical to those described above were conducted with the U373 human glioma cell line and the rat glioma cell lines C6 and F98 with similar results to those reported here for the human glioma cell line U87, indicating that the cytotoxic effects of KM-233 occur irrespective of cell line or species tested (Duntsch et al., 2006).

Cannabinoids are able to promote human U373MG glioma cell death, but only at high concentrations. CB1 is involved in cannabinoid-induced cell death in these cells, insofar as the whole process can be almost totally blocked by incubating the cells with AM251, a specific CB1 receptor antagonist. Additionally, cannabinoids lead to CB1-dependent caspase activation, a hallmark of apoptotic cell death, using these high concentrations. Although cell death is dependent on CB1 receptors' activation, the high concentrations of either Δ^9 -THC or HU-210 needed to promote cell death raise doubts about whether CB receptors constitute relevant molecular therapeutic targets to treat glioblastoma in humans (Widmer et al., 2008). These observations are in accordance with recent data that pharmacologically relevant concentrations of cannabinoids do not promote apoptosis either in primary human glioma samples or in several human glioma cell lines (Held-Feindt et al., 2006). These data are in contradiction to those of Galve-Roperh et al. (2000), who concluded from using rat C6 glioma cells that Δ^9 -THC is able to promote glioma cell death both *in vitro* and *in vivo*. These discrepancies may be due to species differences; for instance, rodent glioma cells express CB2 receptors in addition to CB1, whereas human glioma cells mostly express CB1 and display only modest CB2 levels (Held-Feindt et al., 2006; Widmer et al., 2008), thus explaining the lack of efficacy of cannabinoids in human glioma cells at pharmacologically relevant concentrations (Herrera et al., 2006). In any case, CB1 activation is related to apoptotic cell death in U373MG cells, suggesting that CB1-mediated signalling results in cell death. In this regard, although both ERK and JNK are activated by cannabinoids in U373MG cells, these kinases are unlikely to be involved in cannabinoid-induced cell death, because blocking their activity does not prevent cannabinoid-induced cell death, and, moreover, they are active only at concentrations promoting cell death. Thus, activation of these kinases is more likely to be a consequence than a cause of cannabinoid-induced cell death in U373MG glioma cells. (Widmer et al., 2008).

Different experimental approaches showed that the pro-apoptotic and tumor growth-inhibiting activity of cannabinoids relies on the accumulation of *de novo*-synthesized ceramide, an event that occurs in the endoplasmic reticulum (ER) and eventually leads to execution of cell death via the mitochondrial pathway. A recent study suggested a new link between the two events (Carracedo et al., 2006). They showed that Δ^9 -THC treatment of glioma cells leads to up-regulation of the transcription co-activator p8 and its ER stress-related downstream targets: activating transcription factor 4 (ATF4), CHOP and TRB3. Selective knockdown of ATF4 and TRB3 blocked cannabinoid-induced apoptosis in glioma cells. Inhibition of ceramide synthesis *de novo* prevented Δ^9 -THC-induced p8, ATF4, CHOP and TRB3 up-regulation as well as ER dilation, indicating that ceramide accumulation is an early event in the cannabinoid-triggered ER stress and apoptosis in glioma cells (Carracedo et al., 2006). Another key modulator of apoptosis is the p53 protein. Activation of p53 by phosphorylation can lead to apoptosis, as a result of activation of multiple apoptotic pathways. In cortical neuron cells, p53 is necessary for Δ^9 -THC to induce apoptosis, as shown by abrogating the effects of Δ^9 -THC by using a p53 inhibitor and p53 small interfering ribonucleic acid (siRNA) (Downer et al., 2007). Other studies in neuronal cells also showed that AEA could increase p53 expression/activity through a CB1 mediated pathway involving MAPKs (Pasquariello et al., 2009). Evidence for a negative correlation between TRPV1 expression (mRNA, protein) and disease score was obtained with glioma cells where capsaicin induced apoptosis through an increased calcium influx and p38 activation but not ERK/MAPK activation; these

effects were prevented by the TRPV1 antagonist capsazepine (Amantini et al., 2007). Furthermore, transfection of TRPV2 into glioma cells resulted in reduced cell viability and increased Fas-induced apoptosis (Nabissi et al., 2010). Clearly, activation of TRPV2, like TRPV1, also exerts a negative control on glioma cell survival and proliferation. Such activation could be induced by the cannabinoid/endocannabinoid agonists. Elevated endocannabinoid levels were found in glioblastoma tissue as compared to non-tumor brain tissue, suggesting that endocannabinoid signaling may play a role in negative control of cell divisions (Petersen et al., 2005). In cultured human glioblastoma cells, synthetic CB agonists inhibited proliferation and induced cell death (McAllister et al., 2005). Administration of CB agonists inhibited the expression of matrix metalloproteinase (MMP)-2 in human glioblastoma, an enzyme that revealed a poor prognosis of glioblastoma upon its activation (Blazquez et al., 2008).

In one study it was reported that when CB1 and CB2 antagonists were given alone, they could not reverse the antiproliferative effects of Δ^9 -THC (Galve-Roperh et al., 2000). Only when the two antagonists were used in combination could full reversal of the antiproliferative effects be produced. This suggested that both receptors would need to be activated to limit cell growth. On the other hand, another investigation using rat C6 glioma cells found that either antagonist could partially reverse the antiproliferative effects of Δ^9 -THC (Recht et al., 2001). The data suggest that a common pathway could be activated through either CB1 or CB2 receptors and this leads to inhibition of cell growth and increased cell death (Sanchez et al., 2001).

Cannabinoids promote survival of glial cells and neurons in different models of injury, suggesting that the anti-proliferative effect of cannabinoids is selective for brain tumor cells, while viability of normal brain cells remains unaffected or even favored by cannabinoid challenge (Molina-Holgado et al., 2002; Guzman, 2003). Several mechanisms could be responsible for cannabinoid compounds targeting only the cancer cells, including differences in cannabinoid signaling in glioma and normal neural cells and selective over-expression of the CB2 receptor in tumor cells. In contrast to pro-apoptotic action of Δ^9 -THC and WIN55,12-2 on transformed glial cells, treatment of primary cultured astrocytes with these CB1/CB2-activating cannabinoids does not trigger ceramide generation *de novo* or induction of ER stress-related genes. Negligible CB2 receptor expression in the normal brain and its abundance in high grade gliomas confer a relative safety of CB2-selective agonists for targeted glioma therapy. Moreover, the CB2 selective compounds are devoid of undesirable psychodysleptic side-effects, attributed to marijuana abuse, which are mediated by the CB1 receptor.

Cannabinoids induce cyclooxygenase-2 (COX-2) isoenzyme expression in H4 human neuroglioma cells via a pathway independent of CB or vanilloid receptor activation. The underlying mechanism was recently shown to involve increased ceramide synthesis, which in turn leads to p38 and p42/44 MAPKs activation (Hinz et al., 2004). R(+)-methanandamide (R(+)-MA) has been shown to induce apoptosis of H4 human neuroglioma cells via a mitochondrial-dependent pathway that becomes activated, at least in part, through up-regulation of the COX-2 enzyme (Ramer et al., 2001; Eichele et al., 2006). H4 cells treated with R(+)-MA showed typical signs of mitochondrial apoptosis, i.e., release of mitochondrial cytochrome c into the cytosol and activation of initiator caspase-9. Moreover, activation of the executor caspase-3 was observed following cannabinoid treatment. Cells were fully protected from apoptotic cell death by the caspase-3 inhibitor Ac-DEVD-CHO, indicating a crucial role for caspase-3 activation in R(+)-MA-elicited apoptosis. Furthermore, cleavage of the caspase-3 target protein poly ADP ribose polymerase (PARP) was registered. All of the aforementioned effects were substantially reduced by the selective COX-2 inhibitor celecoxib (1 mM) at a pharmacologically relevant, nonapoptotic concentration (Eichele et al., 2006).

Effects on angiogenesis

Cannabinoids inhibited growth and angiogenesis of gliomas in animal models (Blazquez et

al., 2003). They may further reduce the invasive potential of cancer cells by inhibiting their adhesion to the vascular endothelium. The adhesion molecules (ICAM-1 and VCAM-1), are a prerequisite for extravasation of circulating cells from blood vessels. They have been shown to be downregulated at the mRNA level after treatment with WIN 55,212-2 in astrocytes (Curran et al., 2005).

In rat glioma and human tumor cell lines selective activation of CB2 receptors attenuated malignant cell growth and angiogenesis (Sanchez et al., 2001; Casanova et al., 2003). Moreover, CB agonists evoked a reduction of tumor size by decreasing VEGF production (Blazquez et al., 2004) (thus, inhibiting glioblastoma tumor angiogenesis) the levels of which correlate with the histopathological glioblastoma grade (Samoto et al., 1995; Schmidt et al., 1999). *In vitro* experiments with human umbilical endothelial cells (HUVECs) showed a potential role of cannabinoids also in the proliferation and migration of endothelial cells (Blazquez et al., 2003). These effects, associated to the reduction of VEGF and VEGF receptor-2 (VEGFR-2) levels in glioma cells following cannabinoid administration, might lead to inhibition of angiogenesis in gliomas (Blazquez et al., 2004), thus enhancing direct anti-tumor activity. In addition, the platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) is known to have an important role in vascular biology, including angiogenesis as well as mechanosensing of endothelial cells (Woodfin et al., 2007). An abundant receptor expression was identified on endothelial cells of glioblastoma vessels, in which immunoreactivity for CB2 receptors was enhanced in comparison to CB1 (Schley et al., 2009). Apart from CB1 receptors being abundantly distributed in human brain, CB2 receptors are detected exclusively in perivascular glia cells of the cerebellum (Nunez et al., 2004) and in areas of dense micro-vascularisation of glioblastoma (Ellert-Miklaszewska et al., 2007). The characteristically fast glioblastoma growth is dependent on new blood vessel formation for survival.

Pro-proliferative effects of cannabinoids

As already reported, some cannabinoids may have modest pro-proliferative properties in human U373MG glioma cells. These cells are sensitive only to very high, pharmacologically irrelevant concentrations of cannabinoids, so it seems unlikely that cannabinoids would constitute promising therapeutic molecules since they do not induce glioma cell death at doses that could be applied safely to humans.

Recent reports have demonstrated that Δ^9 -THC and the endocannabinoid methanandamide can stimulate cancer cell growth at concentrations of 100–200 nM (Velasco et al., 2001; Sanchez et al., 2003; Hart et al., 2004; Widmer et al., 2008). A general hypothesis has been proposed that CB receptor activation of short term or low levels vs. long-term and high levels of ERK stimulation is the switch between cell growth and cell death (Velasco et al., 2004). Two pathways have been described that lead to stimulation of cell growth by cannabinoids. The first is linked to direct CB1 and CB2 receptor activation of the PKC-PI3K-ERK pathway and subsequent up-regulation of androgen and nerve growth factor receptors (Velasco et al., 2001; Sanchez et al., 2003). Alternatively, the mitogenic effects of cannabinoids might be the result of tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated trans-activation of the epidermal growth factor receptor leading to ERK stimulation (Hart et al., 2004).

Treatment options and clinical trials

For decades, cannabinoids have been known to exert palliative effects in cancer patients. Δ^9 -THC, the most psychoactive phytocannabinoid, and its synthetic analogue LY109514 (nabilone) are approved for treatment of chemotherapy-induced nausea and emesis. Other potential palliative properties of cannabinoids such as appetite stimulation and analgesia are presently tested in oncology (Guzman, 2003; Zogopoulos et al., 2013). Apart from these actions, a number of plant derived (e.g. Δ^9 -THC and CBD), synthetic (e.g. WIN-55,212-2) and endogenous cannabinoids (e.g. AEA and 2-AG) have been shown to block cancer cell proliferation, induce apoptosis of cancer

cells and have neuroprotective effects both *in vitro* (Sanchez et al., 1998; Jacobsson et al., 2001) and *in vivo* (Galve-Roperh et al., 2000; Casanova et al., 2003), as recently reviewed (Zogopoulos et al. 2013).

Clinical experimental therapies administered intracranially Δ^9 -THC via catheter in the resection cavity of glioblastomas and it was found that Δ^9 -THC inhibited the facilitated tumor growth in glioblastoma patients (Guzman et al., 2006). Δ^9 -THC induced apoptosis in several cancer cell lines but showed less efficacy in nontransformed cell counterparts (Galve-Roperh et al., 2000; Guzman, 2003; McAllister et al., 2005). There is currently an ongoing clinical trial in order to evaluate the safety profile and assess the frequency and severity of adverse events in recurrent glioblastoma patients receiving Sativex (Δ^9 -THC and CBD) in combination with dose-intense Temozolomide (<http://clinicaltrials.gov/show/NCT01812603>). Based on evidence from *in vitro* and *in vivo* preclinical studies, as well as from a pilot phase I clinical trial in patients with recurrent GBM and intratumoral injection of Δ^9 -THC, cannabinoids appear to have a favorable safety profile and do not produce the generalized toxic effects like most conventional chemotherapeutic drugs (Guzman et al., 2006).

DISCUSSION

Cannabinoids, the active components of marijuana and their other natural and synthetic analogues have been reported as useful adjuvants to conventional chemotherapeutic regimens for preventing nausea, vomiting, pain and for stimulating appetite. Furthermore, the use of cannabinoids in cancer therapy as coadjuvants to favour tumor regression is under active research at this moment and may complement in the future the therapeutic profile of their administration (Velasco et al., 2012; Morelli et al., 2014)

The historical median survival of patients with GBM using the best radiological, surgical, and anti-tumor drug therapy available is less than one year depending on age and other prognostic factors (Jemal et al., 2002). Unfortunately, therapeutic adjuvants to surgery such as radiotherapy and chemotherapy provide only a minor improvement in the disease course and life expectancy and are associated with significant side effects (Green et al., 1983; Grant et al., 1995). Current chemotherapy regimens for newly diagnosed malignant glioma include single-agent intravenous BCNU, single-agent oral temozolomide or the combination of procarbazine, chloroethylcyclohexylnitrosourea (lomustine, CCNU) and vincristine (PCV combination). With rare exceptions, to date there have been no good data proving prolongation of time to progression or survival for patients with malignant glioma when other drugs are added to the “standard” BCNU or PCV chemotherapeutic regimens, including cisplatin (Boiardi et al., 1997; Grossman et al., 2000), carboplatin (Levin et al., 1995, 2000; Brandes et al., 1997), dibromodulcitol (Hildebrand et al., 1994), mercaptopurine (Halperin et al., 1996), or 6-thioguanine (Prados et al., 1998). The toxicity profile varies among these treatments, with myelosuppression being the most frequent dose-limiting factor (Nelson et al., 1988; Grant et al., 1995). Recent clinical trials have shown some efficacy for the oral chemotherapeutic agent temozolomide, particularly when given as an adjuvant with concurrent external beam radiotherapy (Stupp et al., 2002). However, two-year survival still remains less than 30% on this regimen, and significant drug-related toxicities occur.

CB1 and CB2 receptors' ligands have recently been shown to have varying degrees of efficacy against a variety of cancer cell lines. In fact, the antitumor effects of Δ^9 -THC, the principal psychoactive component of marijuana, have been known since the 1970s (Munson et al., 1975). Subsequent studies have demonstrated that several plant-derived (Δ^9 -THC and CBD), synthetic (WIN-55, 212-2, JWH-133 and HU-210), and endogenous cannabinoids (AEA and 2-AG) exert antiproliferative actions and induce apoptosis in various mouse, rat, and human cancer cells in culture (Sanchez et al., 1998; Ruiz et al., 1999; Jacobsson et al., 2000).

Clinically investigated cannabinoids, in contrast to conventional cancer chemotherapies, do not exhibit the typical toxicities associated with most chemotherapeutic agents, are well studied and

tolerated by patients, and have the ability to penetrate the blood–brain barrier (BBB). Of particular interest is the report that selective CB2 receptor-activating compounds are effective in regressing gliomas and skin carcinomas while inhibiting pain in the absence of overt signs of psychoactivity (Huffman et al., 1999; Sanchez et al., 2001; McKallip et al., 2002; Guzman, 2003; Blazquez et al., 2004; Valenzano et al., 2005). Given that the unwanted psychotropic effects of cannabinoids are mediated largely by CB1 receptors in the brain, a strategic approach to designing cannabinoids as anti-tumor chemotherapeutics would be the development of selective CB2 receptor agonists.

Psychotropic effects limit the medicinal use of cannabinoids in humans. Although there have been no reported deaths related to Cannabis toxicity (Hall and Solowij, 1998), the acute undesirable side effects include drowsiness, anxiety, depression, palpitations, impaired reaction time, and motor skills with major implications for driving and work-related activities (Adams and Martin, 1996; Hall and Solowij, 1998). The chronic side effects include damage to the respiratory system, impairment of reproductive function, psychosis (in large doses), and possible association with schizophrenia (Turner and Tsuang, 1990; Moore et al., 2007).

A further drawback of using cannabinoids as a potential treatment for glioma is that the high concentrations necessary to promote cell death are very likely to affect neighboring brain cells, such as neurons and astrocytes (Widmer et al., 2008). Cannabis smoking might even constitute a risk factor for developing brain tumors (Efird et al., 2004). Variation in the effects of cannabinoids in different cell lines and tumor models could be due to the differential expression of CB1 and CB2 receptors. Thus, overexpression of cannabinoid receptors may be effective in killing tumors, whereas low or no expression of these receptors could lead to cell proliferation and metastasis because of the suppression of the antitumor immune response. The anti-inflammatory properties of cannabinoids (including Δ^9 -THC) might prevent the immune system from efficiently targeting and destroying cancer cells, thereby potentially promoting tumor growth. Tumor-promoting effects of cannabinoids on glioblastoma cells have been reported (Hart et al., 2004). In this regard, McKallip et al. (2005) have suggested that tumors expressing low levels of CB receptors are not only unresponsive to Δ^9 -THC but are additionally protected from attack by the immune system, in a CB2-dependent manner. It is also reported that low doses of cannabinoid administration accelerate proliferation of cancer cells instead of inducing apoptosis and, thereby, contribute to cancer progression (Sarfaraz et al., 2008).

There is a need for further in-depth studies to elucidate the precise mechanism of cannabinoid action in cancer cells. The safety of Δ^9 -THC administration has been determined and a dose escalation regimen showed that cannabinoid delivery was safe and could be achieved without overt psychoactive effects (Guzmán et al., 2006). In view of the fair safety profile of most cannabinoids together with their antiproliferative action on tumor cells, clinical trials are required to determine whether cannabinoids could be used for the inhibition of tumor growth in a clinical setting. If this could be established, then one can hope that non-toxic, non-habit-forming cannabinoids could be developed as novel therapeutic agents for the treatment of cancer.

CONCLUSION

Even after decades of research aimed at developing effective therapies, the current prognosis remains dismal in high-grade astrocytoma diagnosis (Fisher and Buffler, 2005). Treatments frequently offer a merely palliative therapy that typically includes initial surgical resection of the neoplastic region followed by a combination of intense radio- and chemotherapies. Targeted radiotherapy moderately improves patient outcome (Fisher and Buffler, 2005), increasing mean survival time of patients with grade IV astrocytomas by 22 weeks. Chemotherapeutic regimens often fall short of effectively opposing tumor progression, because of dose thresholds and abbreviated treatment cycles due to the characteristically limited life expectancy after patient diagnosis.

When considering cannabinoids to treat astrocytomas, many have attempted to isolate the

non-psychotropic therapeutic effects ascribed to CB2 receptor activation, while eliminating the psychotropic and addictive properties ascribed to CB1 receptor activation (Sanchez et al., 2001). However, since cannabinoids have outstanding LD50 values (mainly because CB receptors are barely expressed in brain regions that control vegetative functions) (Iversen, 2000), the treatment of tumors with high concentrations of cannabinoids should not be overlooked.

Thus, direct stimulation of the up-regulated CB2 receptors might be explored as an effective therapeutic approach for the treatment of gliomas avoiding CB1 receptor-mediated psychotropic side effects. In addition, CB2 receptor expression might also be a useful tool/marker for the diagnosis of glial cell proliferation and malignancy (De Jesus et al., 2010), although there have been reports that CB2 expression in astrocytoma and glioma tissues did not differ from normal brain tissue (Held-Feindt et al., 2006).

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ABBREVIATIONS

CB1 receptor: cannabinoid receptor type 1

CB2 receptor: cannabinoid receptor type 2

Δ^9 -THC: Δ^9 -Tetrahydrocannabinol

CBD: cannabidiol

CBN: cannabinol

AEA: anandamide

2-AG: 2-arachidonoylglycerol

NADA: N-arachidonoyldopamine

2-AGE: 2-arachidonoylglyceryl ether/noladin ether

OAE: O-arachidonoylethanolamine/virodhamine

AC: adenylate cyclase

ERK: extracellular signal regulated kinase

MAPK: mitogen-activated protein kinase

CNS: central nervous system

TRPA: transient receptor potential ankyrin receptor

TRPM: transient receptor potential melastatin receptor

TRPV: transient receptor potential vanilloid receptor

PPARs: peroxisome proliferator-activated receptors

NF- κ B: nuclear factor-kappa B

GBM: glioblastoma multiforme

NAE: n-acylethanolamine

PI3/PI3K: phosphoinositide-3 kinase

Akt/PKB: protein kinase B

MMPs: matrix metalloproteinases

VEGF: vascular endothelial growth factor

TRB3: pseudo-kinase tribbles homolog 3

CHOP: C/EBP-homologous protein

mTORC1: mammalian target of rapamycin C-1

ER: endoplasmic reticulum

KIP1: cyclin-dependent kinase inhibitor 1

cdks: cyclin-dependent kinases

c-JNK: c-Jun NH₂-terminal kinase

ATF4: activating transcription factor 4

cAMP: cyclic adenosine monophosphate

PKA: protein kinase A

FAAH: fatty acid amide hydrolase
MGL: monoacylglycerol lipase
OTFP: 3-octylthio-1,1,1-trifluoropropan-2-one
EGF: epidermal growth factor
BDNF: brain-derived neurotrophic factor
NGF: nerve growth factor
GFAP: glial fibrillary acidic protein
RT-PCR: reverse transcription polymerase chain reaction
SEGA: subependymal giant cell astrocytoma
IGF1R: insulin-like growth factor 1
EGFR: epidermal growth factor receptor
PDGFR: platelet-derived growth factor receptor
COX-2: cyclooxygenase-2
NF1: neurofibromatosis type-1
GLYT1: glycine transporter 1
PKC- α : protein kinase C-alpha
BCNU: bis-chloroethylnitrosourea or carmustine
SCID: severe combined immunodeficiency
siRNA: small interfering ribonucleic acid
PARP: poly ADP ribose polymerase
ICAM-1: intercellular adhesion molecule 1
VCAM-1: vascular cell adhesion molecule 1
HUVECs: human umbilical endothelial cells
PECAM-1: platelet endothelial cell adhesion molecule-1
TACE: tumor necrosis factor alpha-converting enzyme
CCNU: chloroethyl-cyclohexylnitrosourea or lomustine
BBB: blood-brain barrier

Figure 1: Natural cannabinoids

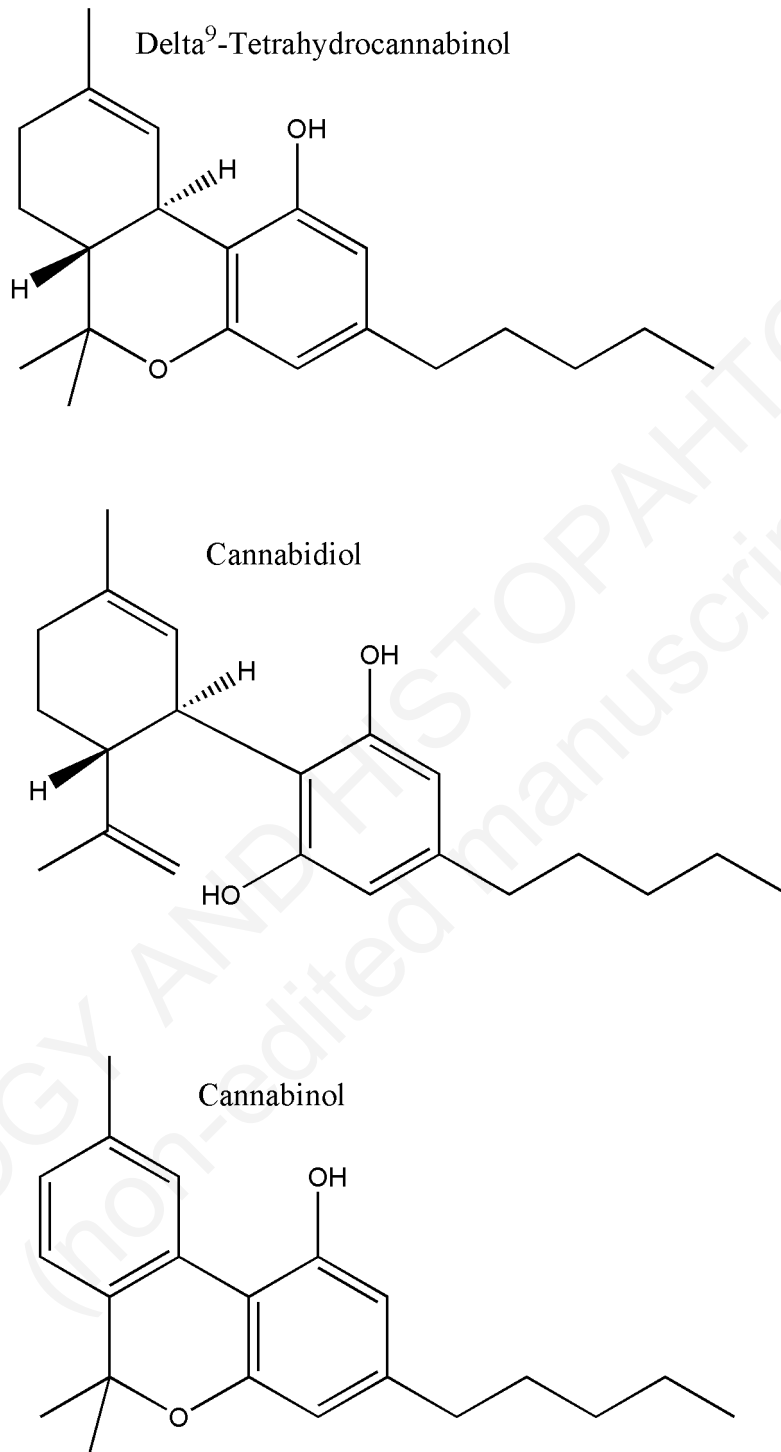
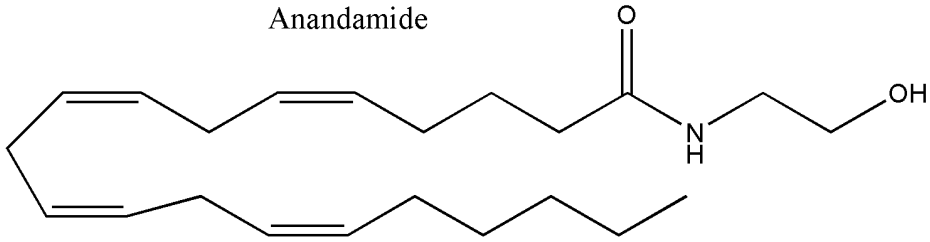
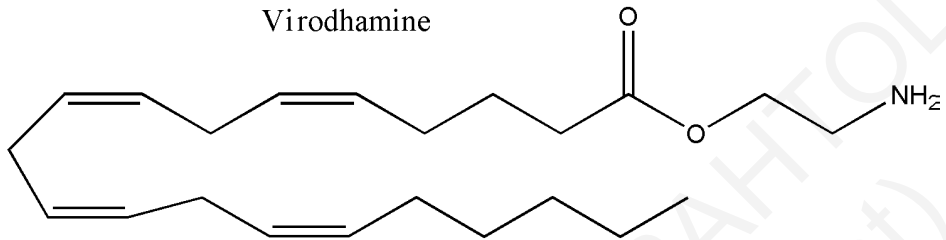


Figure 2: Main endocannabinoids

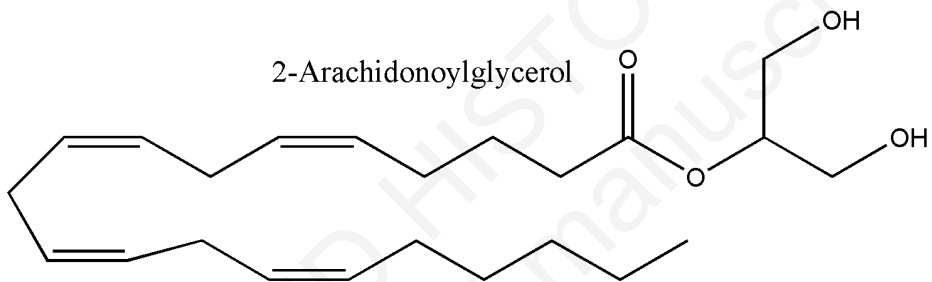
Anandamide



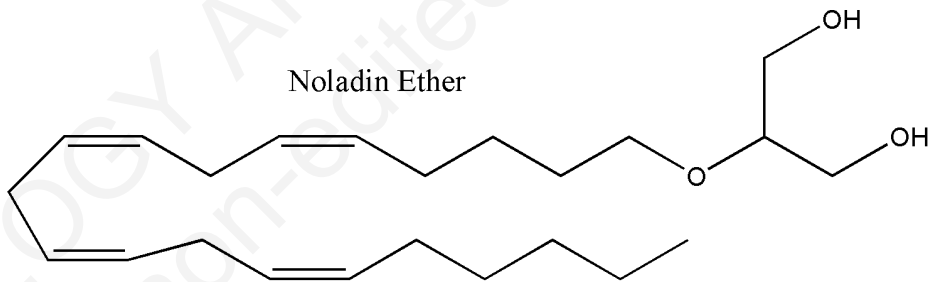
Virodhamine



2-Arachidonoylglycerol



Noladin Ether



N-Arachidonoyldopamine

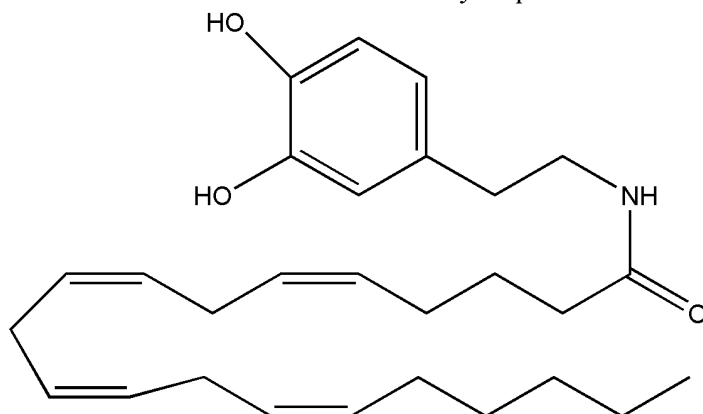


Figure 3: Cannabinoid pathways leading to apoptosis

