
Cannabidiol as potential anticancer drug

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Cannabinoids in the treatment of cancer

Cannabinoids are currently used in cancer patients to palliate wasting, emesis and pain that often accompany cancer. A significant advancement in cannabinoid use in cancer treatment came from the discovery of a potential utility of these compounds for targeting and killing cancer cells. In 1975 Munson *et al.* [17] demonstrated that the administration of Δ^9 -THC, Δ^8 -THC and cannabidiol inhibited the growth of Lewis lung adenocarcinoma cells *in vitro* as well as *in vivo* after oral administration in mice. The interest in anticarcinogenic properties of cannabinoids was even renewed after the discovery of the eCB system and the cloning of the specific cannabinoid receptors. Since then, several cannabinoids have been shown to exert anti-proliferative and pro-apoptotic effects in various cancer types (lung, glioma, thyroid, lymphoma, skin, pancreas, uterus, breast, prostate and colorectal carcinoma) both *in vitro* and *in vivo* [18–26]. Moreover, other antitumorigenic mechanisms of cannabinoids are currently emerging, showing their ability to interfere with tumour neovascularization, cancer cell migration, adhesion, invasion and metastasization [27].

However, the clinical use of Δ^9 -THC and additional synthetic agonists is often limited by their unwanted psychoactive side effects, and for this reason interest in non-psychoactive phytocannabinoids, such as CBD, has substantially increased in recent years. Interestingly CBD has no psychotropic activity and, although it has very low affinity for both CB₁ and CB₂ receptors, it has been recently reported to act with unexpectedly high potency *in vitro* as antagonist of CB₁ receptors in the mouse vas deferens [28] and brain [29] tissues. Additionally, CBD displays inverse agonism at human CB₂ receptors [29]. Moreover, other putative molecular targets of CBD are TRPV, 5-HT_{1A}, GPR55 and PPAR γ receptors (see Figure 2). Besides its beneficial effects in the treatment of pain and spasticity and other CNS pathologies, several reports demonstrated that CBD possesses antiproliferative, pro-apoptotic effects and inhibits cancer cell migration, adhesion and invasion.

CBD and Breast Cancer

In 2006 Ligresti *et al.* [30] demonstrated for the first time that CBD potently and selectively inhibited the growth of different breast tumour cell lines (MCF7, MDA-MB-231), with an IC₅₀ of about 6 μ M, and exhibited significantly lower potency in non-cancer cells. CBD and CBD-rich extracts (containing approximately 70% CBD together with lesser amounts of other cannabinoids) also inhibited the growth of xenografts, obtained by s.c. injection into athymic mice of human MDA-MB-231 cells, and reduced infiltration of lung metastases derived from intrapaw injection of breast carcinoma cells. Among the possible cellular and molecular mechanisms underlying these effects, CBD seemed to involve direct TRPV1 activation and/or CB₂ indirect activation (via FAAH), as well as induction of oxidative stress. Later on, McAllister's group [31] demonstrated that, besides proliferation, CBD also interfered with two other crucial steps of breast cancer cell progression, invasion and metastasization. Among the three different groups of cannabinoid compounds tested (phytocannabinoids with affinity for CB₁ and CB₂ receptors, phytocannabinoids with no appreciable affinity for CB₁ and CB₂ receptors and synthetic compounds with affinity for CB₁ and CB₂ receptors), CBD was shown to be one of the most effective inhibitors of human breast cancer cell proliferation, being equipotent to Δ^9 -THC and CP55940 in inhibiting, respectively, MDA-MB-231 and MDA-MB-436 cell growth, and being the most potent inhibitor of the MDA-MB-231 cell migration. Interestingly, CBD regulated the expression of key genes involved in the control of cell proliferation and invasion through the downregulation of Id-1 expression, an inhibitor of basic helix-loop-helix transcription factors, whose overexpression in breast cancer cells is responsible for proliferation, migration and invasion. Therefore, the ability of CBD to decrease significantly Id-1 expression in breast cancer cells was associated with its efficacy in reducing tumour aggressiveness.

Four years later, the same group [32] demonstrated that the observed effect of CBD on Id-1 expression was mediated by the upregulation of the extracellular signal-regulated kinase phosphorylation (pERK).

Indeed, the ERK inhibitor, U0126, partially reverted CBD-induced inhibition of proliferation and invasion as well as its effect on Id-1 expression. Besides ERK upregulation, also the production of reactive oxygen species (ROS) was shown to mediate CBD-induced effects on cell proliferation and Id-1 expression, since the use of a ROS scavenger (tocopherol) reversed the aforementioned CBD effects. Moreover, these authors demonstrated that CBD was effective in reducing the primary tumour mass and the size and number of metastatic foci *in vivo*.

Finally, the excellent paper of Shrivastava *et al.* [33] adds fresh light on the cellular mechanism through which CBD induces cell death in breast cancer cells. These authors showed that CBD induced a concentration-dependent cell death of both oestrogen receptor-positive and oestrogen receptor-negative breast cancer cells with a mechanism independent of CB₁, CB₂ and TRPV1 receptor activation. Interestingly, the effective concentrations of CBD in tumour cells have little effect on non tumourigenic, mammary cells. Moreover, electron microscopy revealed morphologies consistent with the coexistence of autophagy and apoptosis, these events being promoted by the induction of endoplasmic reticulum (ER) stress and the inhibition of Akt/mTOR/4EBP1 signalling. In addition, CBD-driven increase in ROS production is fundamental to induce both apoptosis and autophagy. Examining further the cellular mechanism involved in CBD-induced cell death, they found that CBD reduced mitochondrial membrane potential, triggered the translocation of the Beclin2 interacting protein (Bid) to the mitochondria and the release of cytochrome C to the cytosol and, ultimately, the activation of the intrinsic apoptotic pathway.

Finally, the relationship between CBD-induced apoptosis and autophagic cell death was explored by blocking each form of cell death with specific inhibitors. Caspase inhibition reduced CBD-induced apoptosis and the expression of protein markers in breast cancer cells, whereas the inhibition of autophagy enhanced apoptosis as a compensatory/alternative mechanism of cell death. The apoptosis : autophagy ratio in CBD-induced cell death seemed to be mediated via Beclin1, a key signalling molecule in the autophagic process. CBD treatment induced the cleavage of Beclin1 and the subsequent translocation of the cleavage product to the mitochondria where it may induce apoptosis through the enhancement of cytochrome C release [34, 35].

As a whole this work highlights the presence of a complex balance between autophagy and mitochondria-mediated apoptosis in CBD-induced breast cancer cell death and strengthens the idea that CBD can be considered as an alternative agent for breast cancer therapy.

CBD and Glioma

CBD also possesses anti-tumoural properties in gliomas, tumours of glial origin characterized by a high morphological and genetic heterogeneity and considered one of the most devastating neoplasms, showing high proliferative rate, aggressive invasiveness and insensitivity to radio- and chemotherapy.

After the seminal paper of Jacobsson *et al.* [36] demonstrating a serum-dependent effect of CBD upon C6 murine glioma cell proliferation, Massi *et al.* in 2004 [37] reported that CBD was effective in inhibiting U87-MG and U373 human glioma cell proliferation *in vitro* through the induction of apoptosis. Interestingly, CBD did not affect viability of non-transformed primary glial cells [38]. When tumour xenografts were generated in immune-deficient mice, *in vivo* intratumoural treatment with CBD significantly reduced tumour growth [37].

The anti-proliferative effect of CBD was cannabinoid and vanilloid receptors independent. The CB₂receptor antagonist SR144528 reverted the effect of CBD, but in a weak and transient manner [37]. More importantly, this paper demonstrated for the first time that the anti-tumour effect of CBD involved the induction of oxidative stress, through increased early production of ROS, depletion of intracellular glutathione and increased GSH-associated enzymatic activity. Accordingly, the CBD anti-proliferative effect was reversed by the anti-oxidant, tocopherol. Importantly, CBD did not induce ROS production in non-transformed primary glial cells [38].

Among the cellular events involved in glioma cell death, CBD produced a time-dependent release of cytochrome C and activation of caspase-8, -9 and -3, suggesting the involvement of both the intrinsic and extrinsic apoptotic pathways [38]. Marcu *et al.* [39] later confirmed the efficacy of CBD in inhibiting the growth of multiple glioblastoma cell lines in a more potent way than Δ^9 -THC. Interestingly, combined treatment of Δ^9 -THC with CBD demonstrated that CBD enhanced the Δ^9 -THC inhibitory effect on glioblastoma cell growth, but not on invasiveness [39]. In line with this, more recently Torres *et al.* [40]

confirmed that combined treatment with CBD and Δ^9 -THC greatly reduced human glioma cell viability enhancing both autophagy and apoptosis and triggering caspase-3 activation. Moreover, combined administration of submaximal doses of CBD and Δ^9 -THC reduced the growth of U87-MG cell-derived tumour xenografts in nude mice to a greater extent than treatment with the individual compounds, suggesting the potential use of combination therapy which would reduce the amount of the psychoactive Δ^9 -THC.

The synergistic effect of combined therapy implied *in vitro* cell cycle arrest, ROS induction and apoptosis through sustained activation of caspases-3, -7 and -9, as well as specific modulation of the extracellular signal-regulated kinase, ERK [39]. These effects were not observed with either compound individually, indicating them as a prerogative of combination treatment. Differently from Marcu's data [39], our recent results (Dr. Valenti, University of Insubria, Varese, pers. comm.) [41] showed that, both in U87-MG and in Δ^9 -THC-resistant T98G human glioma cell lines, CBD *per se* strongly down-regulates two signalling molecules crucial in tumour cell proliferation, ERK and PI3K/Akt. In addition it inhibited the hypoxia-inducible transcription factor HIF-1 α , one of the most critical stimuli for cell survival, motility and tumour angiogenesis. Thus, inhibition of these three molecules appears as part of the multiple molecular targets for CBD anti-neoplastic activity [41].

Further biochemical analysis of glioma tumour tissues excised from nude mice treated *in vivo* with CBD indicated a significant decrease of activity and content of 5-LOX, as well as a marked stimulation of FAAH and a decrease of AEA content [42].

Besides cell growth, CBD reduced glioma cell migration [43] and invasiveness in a Boyden chamber test [39], at concentrations lower than those required to inhibit cell proliferation. The CBD anti-migratory effect was not antagonized by the selective cannabinoid receptor antagonists or by pretreatment with pertussis toxin, indicating no involvement of classical cannabinoid receptors and/or G_{i/o} protein-coupled receptors.

CBD seems to counteract glioma cell proliferation and invasion through multiple mechanisms.

CBD and Leukemia/Lymphoma

Gallily *et al.* [44] provided first evidence of a possible exploitation of CBD in the treatment of lymphoblastic diseases. They demonstrated that CBD treatment induced apoptosis, through caspase-3 activation in human acute myeloid leukaemia HL-60 cell line, whereas it had no effect on human monocytes from normal individuals.

Later on, McKallip *et al.* [45], using the murine EL-4 lymphoma cell line and the human Jurkat and Molt-4 leukaemia cell lines, demonstrated that CBD exposure led to a significant CB₂ receptor-mediated decrease in the number of viable cells as well as to the induction of apoptosis, both *in vitro* and *in vivo*.

In Jurkat cells, CBD exposure resulted in the activation of caspase-8, -9, and -3, the cleavage of poly(ADPribose) polymerase and the decrease in full-length Bid, suggesting a possible cross-talk between the intrinsic and extrinsic apoptotic pathways. Moreover, exposure to CBD led to the loss of mitochondrial membrane potential and subsequent release of cytochrome C. As in other tumour cells, CBD-induced apoptosis required an increase of ROS production. Finally, CBD decreased the levels of phospho-p38 mitogen-activated protein kinase [45], and this effect was blocked by treatment with a CB₂-selective antagonist or ROS scavenger. In addition, CBD treatment caused a significant reduction in tumour burden and increased the level of apoptotic tumours in EL-4-bearing mice [45].

Together, the results suggest that CBD, acting through CB₂ receptors and ROS production, may represent a novel and highly selective treatment for leukaemia. Moreover, previous evidence indicated that human leukaemias and lymphomas expressed significantly higher levels of CB₂ receptors compared with other tumour cell lines, suggesting that tumours of immune origin may be highly sensitive to the CB₂-mediated effects of CBD [46].

CBD and Lung Cancer

Given the poor response of lung cancer to available therapy and its aggressive biological nature, a series of targets and new therapeutic strategies for their treatment are currently being investigated [47–50].

Recently, Ramer *et al.* [51–53] investigated the effect of CBD on the invasive properties of A549 cells, a line of human lung carcinoma cells expressing both CB₁ and CB₂, as well as TRPV1 receptors. Using Matrigel invasion assays, they found a CBD-driven impaired invasion of A549 cells that was reversed by CB₁ and CB₂ receptors as well as TRPV1 antagonists.

CBD treatment concomitantly upregulated the tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) and the CBD-elicited decrease in tumour cell invasiveness was reversed by knocking down TIMP-1 expression through a siRNA approach. These results suggest a causal link between TIMP-1 upregulation and CBD anti-invasive action. CBD was also shown to induce p38 and ERK phosphorylation as upstream mechanisms for TIMP-1 induction and subsequent decreased invasiveness. Interestingly all these cellular events were blocked by cannabinoids or TRPV1 receptor antagonists.

The significant inhibition of A549 cell invasion following CBD treatment was also accompanied by the downregulation of another important factor involved in the regulation of cell spreading, the plasminogen activator inhibitor PAI-1 [52]. CB₁ and CB₂ as well as TRPV1 receptor antagonists suppressed the observed effect of CBD on PAI-1 secretion and cell invasion. Recombinant human PAI-1 and PAI-1 siRNA led to a concentration-dependent up- and down-regulation of invasiveness, respectively, suggesting a crucial role of PAI-1 in A549 invasiveness.

Additionally, *in vivo* studies in thymic aplastic nude mice revealed a significant inhibition of A549 lung metastases following CBD treatment [51] and a significant downregulation of PAI-1 protein was demonstrated in A549 xenografts of CBD-treated rats [52].

It is worth noting that CBD decreased invasiveness in a range of therapeutically relevant concentrations (0.01 to 0.05 μM), since the peak plasma concentrations of CBD in healthy volunteers following administration of Sativex™ (1:1 ratio of Δ^9 -THC and CBD) was reported to be between 0.01 μM to 0.05 μM [54].

Together, these findings provide a novel mechanism underlying the anti-invasive action of CBD on human lung cancer cells and imply its use as a therapeutic option for the treatment of highly invasive cancers.

CBD and Endocrine Tumours

Thyroid cancer is the most common endocrine malignancy and Ligresti *et al.* [30] demonstrated that CBD exerted anti-proliferative effects on rat thyroid KiMol cells, transformed with the v-K-ras oncogene. This effect of CBD was associated with a cell cycle block at the G1/S phase transition, as well as the induction of apoptosis.

Later on, Lee *et al.* [55] demonstrated that CBD markedly induced apoptosis in both murine thymocytes and EL-4 thymoma cells, with CBD-mediated apoptosis occurring earlier in EL-4 cells than in thymocytes. The cellular events triggered by CBD were similar in both T cells, ROS generation playing a pivotal role. The presence of N-acetyl-L-cysteine (NAC), a precursor of glutathione, markedly attenuated the induction of apoptosis and restored the diminished levels of cellular thiols.

The observation that CBD induced oxidative stress in thymocytes, EL-4 cells and splenocytes [56] substantiates the notion that, unlike monocytes, T cells both primary and immortalized, are all sensitive and respond similarly to CBD, with a central role of ROS generation.

CBD and Colon Cancer

Colon cancer is a major cause of morbidity and mortality in Western countries. A recent paper from Izzo's group [57] demonstrated the chemopreventive effect of CBD in a preclinical animal model of colon cancer based on azoxymethane (AOM) administration in mice. AOM treatment was associated with aberrant crypt foci (ACF), polyps and tumour formation, as well as with the upregulation of phospho-Akt, iNOS and COX-2 and the downregulation of caspase-3. CBD was effective in reducing ACF, polyps and tumours and counteracted AOM-induced phospho-Akt and caspase-3 changes. *In vitro* studies, supported the beneficial effect of CBD. Indeed, in colorectal carcinoma cell lines, CBD protected DNA from oxidative

damage, increased endocannabinoid concentrations and reduced cell proliferation in a CB₁-, TRPV1- and PPAR γ -antagonist sensitive manner.

In the light of its safety records, these results suggest that CBD might be worthy of clinical consideration in colon cancer prevention.

CBD and Angiogenesis

Angiogenesis consists of the formation of new blood vessels from pre-existing ones and represents another promising therapeutic target for cancer therapy. Collectively, cannabinoids have been demonstrated to act as anti-angiogenic factors by disposing tumour cells to decrease the production of pro-angiogenic factors and/or by direct modulation of endothelial cells [27].

Surprisingly, so far no study has investigated the effect of CBD on angiogenesis. Our data currently awaiting publication [58] demonstrated that CBD potently inhibited HUVE cells proliferation, migration and invasion through the induction of endothelial cell cytostasis without triggering apoptosis. Interestingly, CBD also affected endothelial cell differentiation into tubular capillaries as well as the outgrowth of capillary-like structures from HUVEC spheroids *in vitro*. In addition, the anti-angiogenic properties of CBD were demonstrated also *in vivo*, using a matrigel sponge model. These effects were associated with down-modulation of several molecules associated with angiogenesis, including MMP2, MMP9, uPA, endothelin-1, PDGF-AA and CXCL16.

Collectively, these preliminary data demonstrate that, besides its well known pro-apoptotic anti-proliferative and anti-invasive actions, CBD may also exert anti-angiogenic effects, thus further strengthening its potential application in cancer therapy.

Conclusion and Future Directions

Collectively, the non-psychoactive plant-derived cannabinoid CBD exhibits pro-apoptotic and anti-proliferative actions in different types of tumours and may also exert anti-migratory, anti-invasive, anti-metastatic and perhaps anti-angiogenic properties. On the basis of these results, evidence is emerging to suggest that CBD is a potent inhibitor of both cancer growth and spread.

Interestingly, the anticancer effect of this compound seems to be selective for cancer cells, at least *in vitro*, since it does not affect normal cell lines. The efficacy of CBD is linked to its ability to target multiple cellular pathways that control tumourigenesis through the modulation of different intracellular signalling depending on the cancer type considered. The most common effect of CBD is the increase in ROS production that seems to be determinant for triggering its beneficial action in all the considered cancer cell types. The role of cannabinoid/vanilloid receptors in mediating CBD effects is more controversial. In some cases (lung, leukaemia, colon) a clear contribution of these receptors has been demonstrated through the use of specific antagonists, but in other cancer types (glioma and breast) their relevance appears only marginal or absent. Besides the *in vitro* data, the efficacy of CBD in reducing tumour growth and, in some cases, metastasization was confirmed in experimental animal models. However, the potential clinical application of CBD for cancer therapy needs some consideration. Its low toxicity is certainly a good starting point. CBD behaves as a non toxic compound; indeed oral administration of CBD 700 mg day⁻¹ for 6 weeks did not show any overt toxicity in humans [59] suggesting its possible exploitation for prolonged treatment. The route of administration appears more problematic since CBD oral absorption is slow and unpredictable. However, 6 weeks of oral CBD treatment 10 mg kg⁻¹ day⁻¹ provoked a mean plasma concentration of CBD between 6 and 11 ng ml⁻¹ (about 0.036 μ M) [60] that did not differ significantly over the 6 weeks of administration. Interestingly, this range of concentration was demonstrated to be active in inhibiting lung cancer cell invasion [52, 53], thus suggesting that in some cases the oral route could be the appropriate choice. Additionally, experimental data showed that combined treatment with CBD and Δ^9 -THC could be more effective in reducing cancer cell proliferation, suggesting that the co-administration may represent a better choice for cancer therapy. Accordingly, oromucosal treatment with Sativex™ 10 mg (a formulation with a 1:1 ratio of CBD and Δ^9 -THC, recently approved for multiple sclerosis) resulted in a CBD plasma concentration of effective in reducing lung cell invasion *in vitro*. Thus, the results obtained with Sativex™ suggest that the use of different associations of phytocannabinoids in a variable proportion might lead to a better outcome without pharmacokinetic interaction [61]. Moreover, oromucosal

administration may represent a first choice in the presence of nausea and vomiting. Finally, the use of CBD/Sativex can be suggested in combination with classical chemotherapeutic agents to check for the presence of a synergistic effect that might potentially allow clinical chemotherapeutic dose reduction, thereby reducing toxicity while maintaining efficacy. In the light of its safety record and considering that CBD is already currently used in patients with multiple sclerosis, the findings here summarized suggest that CBD might be worthy of clinical consideration for cancer therapy.