The Role of Cannabinoids in Cancer: Perspectives Beyond Pain and Palliative Care

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Abstract

Cannabinoids have been studied extensively regarding their analgesic effects for severe pain as well as in palliative care. However, there has been little focus on the potential anticancer effects of cannabinoids. With the rise of the internet and social media, there is enthusiastic interest from patients regarding cannabinoids as a treatment for cancer which can affect their treatment choices. This essay aims to highlight what cannabinoids are and how the endocannabinoid system interacts with the process of tumour formation. Furthermore, this essay will draw from preclinical data to examine the proposed roles and mechanisms of cannabinoids in apoptosis, immune responses, angiogenesis and metastasis. Finally, this essay will briefly touch on what we know from clinical trials and summarise what trials are currently ongoing.
What are cannabinoids?

Cannabinoids are ligands which are specific to receptors of the cannabinoid class, they are lipophilic molecules. There are three main groups which cannabinoids can be classed in; endocannabinoids, phytocannabinoids and synthetic cannabinoids.

Endocannabinoids are part of the physiological endocannabinoid system and are the endogenous ligands of cannabinoid 1 and cannabinoid 2 receptors (CB1 and CB2) (Matsuda et al. 1990, Munro et al. 1993). The most well-known endocannabinoids are arachidonoylglycerol (2-AG) and anandamide (AEA). CB1 and CB2 receptors are G protein coupled receptors which act via inhibition of the adenylyl cyclase pathway leading to downstream effects modulating protein kinase B (Akt), the mitogen-activated protein kinase pathway (MAPK), the cyclooxygenase-2 pathway (COX-2) and the phosphoinositide 3-kinase pathway (PI3K) (Javid et al. 2016, Pertwee et al. 2010). CB2 is more abundant on immune cells whereas CB1 is the main receptor for activation of cannabinoid effects and can be found in high abundance in the central nervous system which explains why cannabinoids have a psychoactive effect (Atwood et al. 2010, Fernandez-Ruiz et al. 2007, Velasco et al. 2012.)

Beyond the neuromodulatory functions of the endocannabinoid system, it has been shown to play a role in immunity, metabolism, ovulation and vascular tone (Katona et al. 2008, Pertwee et al. 2009).

Phytocannabinoids are the metabolites of the Cannabis plant and there have been many discovered, the most researched are tetrahydrocannabinol (THC) and cannabidiol (CBD). THC acts via CB receptors in the body but is highly psychoactive, causing effects of euphoria; it also displays anti-emetic, anti-inflammatory and analgesic properties (Kramer et al. 2015, Pertwee et al. 2010). In comparison CBD acts via other receptors such as G protein coupled receptor 55 (GPR55), transient receptor potential channel subfamily V member 1 (TRPV1) and peroxisome proliferator-activated receptors (PPARs). CBD has anti-anxiety effects and reduces the effects of THC on the CB1 receptors in the brain (Kramer et al. 2015, Pertwee et al. 2010).

Synthetic cannabinoids are those produced by pharmaceutical companies to achieve a therapeutic effect by interacting with the endocannabinoid system. Examples of these are the drugs nabilone and dronabinol used in the USA – both drugs are THC synthetic analogues which are used alongside chemotherapy to act as an anti-emetic. Another such drug is nabiximols which is an oromuscosal spray containing mostly CBD and is used to treat muscle spasticity in multiple sclerosis (Whiting et al. 2015).

The endocannabinoid system and cancer
There have been many studies examining the link between the endocannabinoid system and cancer, however, the exact role remains unclear. Many studies have shown that there is an upregulation of CB receptors and increased endocannabinoid expression in cancer cell lines including, skin, colon, prostate, liver, endometrium, adenomas, glioblastoma and meningioma (Javid et al. 2016, Fernandez-Ruiz et al. 2007, Sarfaraz et al. 2008, Guzman et al. 2006). This suggests that the endocannabinoid system may be protumorigenic and the cancer cell lines are over expressing aspects of the system to grow.

However, antitumorigenic evidence comes from studies showing that enzymes which breakdown endocannabinoids are found to be upregulated in cancer cell lines and tumours, which suggests that tumours are overcoming any antitumorigenic effects of the endocannabinoid system by producing enzymes to degrade endocannabinoids (Nomura et al. 2010, Thors et al. 2010). Further evidence of the antitumorigenic properties comes from a study of intestinal adenomas in the murine model, silencing of CB1 caused an increase in the growth of adenomas and this was halted by reactivation of CB1 (Wang et al. 2008).

Additionally, it was found that decreasing levels of enzymes which degrade endocannabinoids caused a decrease in tumour growth rate; the elevated level of endocannabinoids has been shown to reduce the number of lesions in the murine colon model of cancer (Izzo et al. 2008). Furthermore, endocannabinoids used to treat prostate cancer in cell lines have shown a dose dependent relationship in inhibition of cell growth (Orellana-Serradell et al. 2015). These findings suggest that there is a direct correlation between endocannabinoid use and growth inhibition in prostate cancer cell lines.

**Effects of cannabinoids: apoptosis and cell viability**

Initial studies into the antiproliferative aspect of cannabinoids were carried out in lung adenocarcinoma cell lines and in murine models via oral intake, these studies showed that cannabinoids may have an antiproliferative effect (Munson et al. 1975). Further data examining the roles of CB1 and CB2 have shown that these receptors stimulate apoptosis in glioma cell lines via accumulation of ceramide (Glave-Roperh et al. 2000, Gomez del Pulgar et al. 2002).

The process by which cannabinoids may produce antiproliferative effects has been suggested as an autophagic mechanism; this was demonstrated in studies which showed that inhibition of autophagy prevents cannabinoid induced apoptosis whereas inhibition of apoptosis does not prevent cell death but not autophagy. This was shown concretely in cell lines of glioma, melanoma, liver and pancreatic cancer where cannabinoids induced autophagy (Salazar et al. 2009, Armstrong et al. 2015, Carracedo et al. 2006, Vara et al. 2011).
A further mechanism which as been suggested in contributing to cell viability in cancer cell lines is related to cannabinoid induced endoplasmic reticulum (ER) stress, this leads to AMP-activated protein kinase activation which activates the calmodulin-dependent protein kinase kinase 2 which then contributes to the cannabinoid induced autophagy mediated cell death (Carracedo et al. 2006).

Moreover, yet another observed mechanism of cannabinoid induced cell death is found from research into breast and skin cancer cell lines. Studies show that cannabinoids induce inhibition of Akt signalling leading to activation of p21 and p27 which are proteins that inhibit the cyclin-dependent kinases, these proteins phosphorylate retinoblastoma protein and cause apoptosis by blocking the cell cycle (Caffarel et al. 2006, Blazquez et al. 2006, Caffarel et al. 2008). In both glioma and prostate cancer cell lines, a similar effect has been seen due to cannabinoids downregulating Akt signalling. The downregulation can lead to a reduction in phosphorylation of Bcl-2 associated death promoter which ultimately results in apoptosis (Ellert-Miklaszewska et al. 2005, Sarfaraz et al. 2006).

Research into the antiproliferative effects of CBD has been unable to pinpoint an exact mechanism, however, CBD can induce autophagic cell death. Research into neuroblastoma, glioblastoma, melanoma, leukaemia, colorectal, breast, lung and prostate cancer cell lines has shown that CBD reduces the viability of these cells (Armstrong et al. 2015, Singer et al. 2015, De Petrocellis et al. 2013, Borrrelli et al. 2014, Fisher et al. 2016, Elbaz et al. 2015, Ramer et al. 2013, Kalenderoglou et al. 2017). The most researched theories proposed on the mechanism behind this cell death circles around a CB receptor independent stimulation of reactive oxygen species production (McAllister et al. 2015, Shrivastava et al. 2011, Singer et al. 2015, De Petrocellis et al. 2013). Furthermore, research into the effects of CBD on enzymes has shown that CBD inhibits fatty acid amide hydrolase (FAAH) which breaks down endocannabinoids. Therefore, the antiproliferative effects of CBD may be due to the indirect upregulation of the endocannabinoid system (Watanabe et al. 1996, Bisogno et al. 2001). It is unclear which receptors are mediating the CBD responses, however, research has shown that CBD and cannabigerol (CBG) are both potent antagonists of TRPM8 receptors which may be the mechanism behind apoptosis (Pertwee et al. 2010, Borrrelli et al. 2014). Additionally, studies have shown that CB2 may be directly or indirectly involved in the antiproliferative effects of CBD (Ligresti et al. 2006).

Interestingly, the apoptotic actions of cannabinoids appear to be concentration dependent and biphasic. This means that low concentrations in the nanomolar range appears to stimulate growth in vitro and micromolar concentrations appear to inhibit proliferation. Furthermore, the concentration required to reach inhibitory levels far exceeds that seen in the blood of recreational cannabis users (Croxford et al. 2005). Studies have shown that nanomolar doses of THC in cancer cell lines cause
proliferation via metalloprotease and epidermal growth factor receptor (EGFR) leading to upregulation of kinases and growth of the cancer cell, the doses used in the cell lines correspond to those found in serum of recreational users. This data suggests that simply using cannabis recreationally, specifically containing THC, may in fact be detrimental to survival in cancer patients (Hart et al. 2004).

**Effects of cannabinoids: immune system**

It is thought the immune system is involved in the oncogenesis process of a variety of different cancers. This is due to variations in cytokine levels, for example, IL-2 and IFN-γ increase the Th1 response which is cell mediated whereas IL-5 and IL-4 increase the Th2 response which is humorally mediated. Furthermore, IL-10 can suppress the cell mediated immune response; research has suggested that the Th1 cell mediated response is an effective response to cancerous cells (Nishimura et al. 2000). Regarding cannabinoids, most of the research has been focused on CB2 as this receptor is expressed on immune cells, specifically on neutrophils, NK cells, T cells, B cells and monocytes; research has shown that CB2 expression on immune cells correlated with activation of the immune cells (Börner et al. 2008, Croxford et al. 2005).

The impact of CB2 activation in immune cells has been studied. Phytocannabinoids which bind to CB2 impact on both the Th1 and Th2 responses; THC reduced the production of IFN-γ which causes a change from Th1 to Th2 responses and therefore a suppression of T cell production. CBD has been shown to indirectly interfere with CB receptor agonists thereby inhibiting immune cell responses. These findings suggest that cannabinoids appear to have an anti-inflammatory and immunosuppressive effect on the immune system (Cabral et al. 2005, Jan et al. 2003, Yuan et al. 2002, Walter et al. 2003, Eisenstein et al. 2015).

Specific studies into cannabinoids impact on the immune system in cancers found that most of the effects are seen through interactions with T cells and TGF-β production. The presence of cannabinoids leads to a switching from Th1 to Th2 T helper cells which causes an increase in IL-10 and TGF-β and a decrease in IL-2 and IFN-γ; this causes an anti-inflammatory response. Furthermore, this response appears to interfere with the host antitumour immunity (Croxford et al. 2005, Eisenstein et al. 2015, Börner et al. 2009, McKallip et al. 2005). The mechanism proposed is due to cannabinoids causing an upregulation of Th2 gene and a downregulation of Th1 genes via histone modifications. Moreover, studies have shown that cannabinoids can cause immunoglobulin class switching from IgM to IgE which is switch towards Th2 responses. Cannabinoids have been shown to suppress production of proinflammatory cytokines (Croxford et al. 2005, Yang et al. 2014, Agudelo et
al 2008). These findings suggest that cannabinoids have an anti-inflammatory effect on the body, however, these effects may not be beneficial in halting oncogenesis.

Research has been conducted on animal models to examine the effect of THC on the immune response. A study on a murine lung cancer model found that immune responses against cancer were suppressed by THC causing an overall growth of the tumour; it was observed that there was an increase in IL-10 and TGF-β whereas IFN-γ decreased (Zhu et al. 2000). These findings suggest that THC caused an anti-inflammatory response and a switch from Th1 to Th2, which is detrimental to the anticancer immune response. Furthermore, the administration of antibodies against IL-10 and TGF-β reversed the THC induced tumour growth effects. These effects were also blocked by CB2 antagonists. However, the growth of lung cancer in a murine model with severe combined immunodeficiency showed no effects when THC was administered (Zhu et al. 2000). These findings suggest that the immune effects of THC are carried out via IL-10 and TGF-β, CB2 and indirectly using the hosts own immune system; therefore, THC is acting as a modulator of the intact immune system.

A similar study was carried out looking at THC-induced immunosuppression in the murine mammary cancer model. THC exposure led to a growth in the tumour and increased invasiveness. The changes which were seen include an increase in IL-10 and IL-4 which supports the previous evidence of a Th1 to Th2 shift in immune response. This study also demonstrated that Th1 related genes became downregulated and Th2 related genes became upregulated. Furthermore, this study also showed that the effects were mediated by CB2 and that introduction of antibodies reversed the THC immunosuppressive effects (McKallip et al. 2005). These findings support the evidence that THC inhibits the anticancer immune responses.

As CB receptor expression varies from tissue to tissue, it is entirely possible that some tissues and therefore some types of cancers may be less receptive to any anti-cancer effects of cannabinoids. This factor coupled with the THC effects of immunosuppression may in fact promote tumour growth and provide a beneficial environment. The immunosuppression may simply be a stronger response than any anticancer response of cannabinoids and therefore this could be a strong contraindication for medicinal use in cancer patients. However, there is contradictory evidence, as studies have found that cannabinoids also act on the immune system to induce anti-cancer effects. A study examining lung cancer cell lines found in vitro evidence that cannabinoids modulate the expression of intercellular adhesion molecule 1 (ICAM-1) to induce lysis of cancerous cells via the action of lymphokine-activated killer (LAK) cells. This effect was unique to cancer cell lines and was absent in normal bronchial cells (Haustein et al. 2014). Furthermore, inflammatory responses have been associated with the formation of cancerous cells, therefore the anti-inflammatory actions of
cannabinoids may be protective. A study in rats showed that THC administration over a two-year period decreased spontaneous tumour incidence and improved overall survival rates (Chan et al. 1996, Burstein et al. 2009, Liu et al. 2010). These effects may be due to the anti-inflammatory effects of THC; however, it is important to note that this study was carried out on rats and therefore the results may not be easily transferable to humans.

**Effects of cannabinoids: inhibition of angiogenesis and metastasis**

Beyond the interactions with cancer formation described above, cannabinoids have been found to play a role in the invasive aspects of cancer formation, such as angiogenesis and metastasis. Research into the role of cannabinoids in angiogenesis has found that cannabinoids can be inhibitory via their action on vascular endothelial growth factor (VEGF); cannabinoids caused a downregulation in VEGF receptor 1 and 2, this was observed in glioma, skin and thyroid cancer cell lines (Casanova et al. 2003, Blázquez et al. 2004, Portella et al. 2003). Furthermore, cannabinoids appear to trigger apoptosis not only in cancer cell lines as described earlier but also highly proliferative cells such as endothelial cells (Blázquez et al. 2003, Pistani et al. 2007). This action combined with their actions on VEGF leads to an impairment in the development of intratumoural vasculature. Supportive evidence comes from a study which pharmacologically blocked ceramide synthesis, which is formed in response to cannabinoids, this caused a decrease in the observed inhibitory effects on VEGF by cannabinoids (Blázquez et al. 2004). These findings suggest that cannabinoids could play a key role in preventing angiogenesis and therefore reducing the invasiveness of tumours.

Animal models and in vitro breast, lung, cervical and glioma cancer cell lines have been shown to have reduced metastasis when exposed to cannabinoids (Blázquez et al. 2008, Grimaldi et al. 2006, Qamri et al. 2009, Preet et al. 2008, Ramer et al. 2008). A key component of the invasiveness of tumours and metastasis are metalloproteases; studies have suggested that modulation of metalloproteases by cannabinoids may reduce their effects and therefore reduce tumour invasiveness. Research suggests that the mechanism behind these effects is cannabinoids interacting with matrix metalloproteases and tissue inhibitor of metalloproteinases 1 (TIMP-1) (Blázquez et al. 2008, Ramer et al 2008). Interestingly, inhibition of ceramide synthesis once again blocked the effects of reduced metastasis and expression of p8 protein also caused a decrease in the anti-invasive effects (Blázquez et al. 2008). Furthermore, research into lung cancer cell lines has found that CBD caused a reduction in invasiveness via increases in ICAM-1; this mechanism has been linked to modulation of TIMP-1 (Ramer et al. 2012). These findings suggest that cannabinoids reduce invasiveness via modulation of metalloproteases; these effects are dependent on ceramide and interaction with the p8 pathway.
Finally, research conducted on FAAH (the enzyme which breaks down endocannabinoids) has found that inhibition of FAAH causes a decrease in invasiveness via a TIMP-1 dependent mechanism, higher levels of endocannabinoids can be found in lung cancer cell lines which have FAAH inhibited. Therefore, it is possible that the reduction in invasiveness is due to an increase in the endocannabinoids within the cell (Winkler et al. 2016).

**Clinical trials**

Most of the data into the effects of cannabinoids on cancer is from cell lines or animal models. The first phase one clinical trial conducted on humans was a study on nine patients with recurrent glioblastoma multiforme which was resistant to standard treatment. THC was administered to the patients intratumorally and there was no obvious psychactivity shown. Patients displayed a decrease in tumour growth rate and examination of the tumour showed that the effects are in line with preclinical data; there was a reduction in proliferation and increased apoptosis. However, as the sample size had only nine patients it is difficult to draw conclusions beyond the potential safety of the treatment. Overall, the treatment was not disease modifying and the prognosis did not change (Guzman et al. 2006). Further and more robust clinical trials are required to identify any beneficial effects of cannabinoids in the treatment of cancer.

There are current ongoing clinical trials examining the effects of cannabinoids on cancer, however, there is no data currently available. One trial is studying the safety of nabiximol in combination with chemotherapy for recurrent glioblastoma multiforme (NCT01812603, NCT01812616). Another study is examining the effects of CBD on solid tumours (NCT02255292). Further studies are looking into the effects of dexanabinol in healthy controls as well as patients with solid tumours and brain cancer (NCT01489826, NCT01654497, NCT02054754).

**Conclusion**

Cannabinoids are a collection of substrates that bind to CB receptors, they can be found endogenously, in plants and made synthetically. Research into the endocannabinoid system has found that tumours actively try to overcome the endocannabinoid system by increasing FAAH production, and links have been made between the endocannabinoid system and reductions in tumour formation in animal models. Upon further investigation it was found that cannabinoids stimulate cell death via autophagy and cell cycle arrest via indirect interactions with these systems. The apoptotic and antiproliferative effects of cannabinoids were found to be biphasic in nature, with very high doses required to induce the anticancer effects; doses which cannot be easily achieved via
inhalation or oral consumption of the cannabis plant. Furthermore, cannabinoids appear to reduce Th1 immune responses and induce Th2 responses. Th1 responses are protective against cancer, thus cannabinoids may create a protumorigenic environment. However, cannabinoids have also been shown to cause cancer cell lysis via ICAM-1 and LAK cells. Cannabinoids have been shown to clearly have inhibitory effects on angiogenesis via VEGF inhibition; they have also been shown to reduce metastasis via modulation of metalloprotease. Despite these promising preclinical studies, there is very little clinical data on the effects on cannabinoids and further clinical trials are required with some ongoing right now. Further research is needed regarding overcoming the immunosuppressive effects of cannabinoids to gain value from the antiproliferative effects. Additionally, further data is required to understand the best route of administration may be in human subjects, as well as the safety and efficacy of cannabinoids in humans.
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