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Mini-review

Hypothesis: cannabinoid therapy for the treatment of gliomas?

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Abstract

Gliomas, in particular glioblastoma multiforme or grade IV astrocytoma, are the most frequent class of malignant primary brain tumours and one of the most aggressive forms of cancer. Current therapeutic strategies for the treatment of glioblastoma multiforme are usually ineffective or just palliative. During the last few years, several studies have shown that cannabinoids—the active components of the plant *Cannabis sativa* and their derivatives—slow the growth of different types of tumours, including gliomas, in laboratory animals. Cannabinoids induce apoptosis of glioma cells in culture via sustained ceramide accumulation, extracellular signal-regulated kinase activation and Akt inhibition. In addition, cannabinoid treatment inhibits angiogenesis of gliomas in vivo. Remarkably, cannabinoids kill glioma cells selectively and can protect non-transformed glial cells from death. These and other findings reviewed here might set the basis for a potential use of cannabinoids in the management of gliomas.

Keywords: Glioma; Glioblastoma multiforme; Cannabinoid; Apoptosis; Angiogenesis; Ceramide

1. Introduction

Gliomas are defined as those tumours that display histological, immunohistochemical and ultrastructural evidence of glial differentiation. The World Health Organization classifies gliomas according to their cellular features (i.e. resembling astrocytes, oligodendrocytes or ependymal cells) and their grade of malignancy (from I to IV) (Kleihues et al., 2002). Glioblastoma multiforme (GBM), or grade IV astrocytoma, is the most frequent class of malignant primary brain tumours and one of the most aggressive forms of cancer. As a consequence, survival after diagnosis is normally just 6–8 months (Maher et al., 2001). This dramatic behaviour is mainly due to the high invasiveness and proliferation rate of GBM. In addition, GBM exhibits a high resistance to common chemotherapy and radiotherapy. These malignant characteristics may be related to the varying mutations frequently found in these tumours that impact different key pathways involved in the control of cell proliferation, cell survival and DNA repair (Maher et al., 2001; Merlo, 2003).

Current therapeutic strategies for the treatment of GBM and other malignant brain tumours are usually inefficient or just palliative, and include surgery and radiotherapy. Some chemotherapeutic agents such as temozolomide, carmustin, carboplatin and thalidomide have been tested, and the most recent strategies for GBM treatment are focused on gene therapy, but no trial performed to date has been significantly successful (Maher et al., 2001; Louis et al., 2002). It is therefore essential to develop new therapeutic strategies for the management of GBM, which will most likely require a combination of therapies to obtain significant clinical results. Here, we summarize the antiproliferative actions of a family of compounds that are being tested in the laboratory for the management of gliomas: the cannabinoids.

2. Cannabinoids and their receptors

The hemp plant *Cannabis sativa* produces approximately 60 unique compounds known as cannabinoids,

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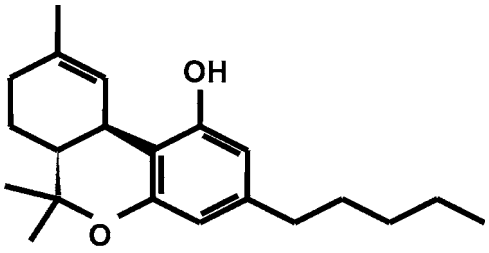
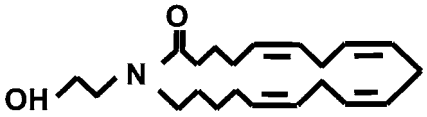
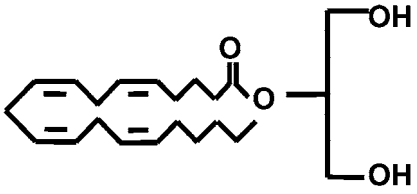


Δ^9-Tetrahydrocannabinol



Anandamide
(arachidonoylethanolamide)



2-Arachidonoylglycerol

Fig. 1. Chemical structure of Δ^9-tetrahydrocannabinol and the endocannabinoids anandamide and 2-arachidonoylglycerol.

of which Δ^9-tetrahydrocannabinol (THC) is the most studied owing to its high potency and abundance in cannabis (Gaoni and Mechoulam, 1964) (Fig. 1). THC exerts a wide variety of biological effects by mimicking endogenous substances—the endocannabinoids anandamide and 2-arachidonoylglycerol (Fig. 1)—that bind to and activate specific cannabinoid receptors (Box 1). So far, two cannabinoid-specific $G_{i/o}$ protein-coupled receptors, CB_1 and CB_2, have been cloned and characterized from mammalian tissues (Howlett et al., 2002). Most of the effects of cannabinoids rely on CB_1 receptor activation. This receptor is particularly abundant in discrete areas of the brain, but is also expressed in peripheral nerve terminals and various extra-neural sites such as testis, eye, vascular endothelium and spleen. In contrast, the CB_2 receptor is almost exclusively present in the immune system (Howlett et al., 2002).

Extensive molecular and pharmacological studies have demonstrated that cannabinoids inhibit adenylyl cyclase through CB_1 and CB_2 receptors. The CB_1 receptor also modulates ion channels, inducing, for example, inhibition of N- and P/Q-type voltage-sensitive Ca^{2+} channels and activation of G protein-activated inwardly rectifying K^+ channels (Howlett et al., 2002). Besides these well-established cannabinoid receptor-coupled events, cannabinoid receptors also modulate several signalling pathways that are more directly involved in the control of cell proliferation and survival, including extracellular signal-regulated kinase (ERK) (Bouaboula et al., 1995), c-Jun N-terminal kinase and p38 mitogen-activated protein kinase (Liu et al., 2000; Rueda et al., 2000), phosphatidylinositol 3-kinase (PI3K)/Akt (Gómez del Pulgar et al., 2000), focal adhesion kinase (Derkinderen et al., 1996) and the sphingomyelin cycle (Sánchez et al., 2001b).

Box 1

The endocannabinoid system

The endogenous cannabinoids, or endocannabinoids, together with their specific CB_1 (Matsuda et al., 1990) and CB_2 (Munro et al., 1993) receptors and their processes of synthesis (Di Marzo et al., 1994; Stella et al., 1997), uptake (Beltramo et al., 1997) and degradation (Cravatt et al., 1996; Dinh et al., 2002), constitute the so-called endogenous cannabinoid or endocannabinoid system. The first endocannabinoid was described by Mechoulam and coworkers (Devane et al., 1992). It was named anandamide (arachidonoylethanolamide), from the sanscrit ananda, "internal bliss", and making reference to its chemical structure (the amide of arachidonic acid and ethanolamine). Subsequently, other endocannabinoid receptor ligands such as 2-arachidonoylglycerol have been described (Mechoulam et al., 1995; Sugiura et al., 1995). These compounds, although structurally not related to THC, bind specifically to cannabinoid receptors and mimic the effects of exogenous plant-derived and synthetic cannabinoids. Endocannabinoids act as retrograde neuromodulatory mediators that, by altering neuronal plasma-membrane Ca^{2+} and K^+ fluxes, inhibit the release of neurotransmitters such as glutamate, dopamine and GABA (Wilson and Nicoll, 2001; Piomelli, 2003). This allows the endocannabinoid system exert control on a wide variety of biological processes such as movement, pain and memory (Piomelli, 2003).

3. Antitumoural action of cannabinoids in gliomas

Cannabinoids have been known for several decades to exert palliative effects in cancer patients, and nowadays capsules of THC (dronabinol, Marinol) and its synthetic analogue LY109514 (nabilone, Cesamet) are approved to treat nausea and emesis associated with cancer chemotherapy (Tramer et al., 2001). In addition, several clinical trials are testing other potential palliative properties of cannabinoids in oncology such as appetite stimulation and analgesia (Guzmán, 2003). Apart from these palliative actions, a number of plant-derived (for example, THC and cannabidiol), synthetic (for example, WIN-55,212-2 and HU-210) and endogenous cannabinoids (for example, anandamide and 2-arachidonoylglycerol) have been shown to exert antiproliferative actions on a wide spectrum of tumour cells in culture (Bifulco and Di Marzo, 2002; Guzmán, 2003). More importantly, cannabinoid administration to nude mice curbs the growth of various types of tumour xenografts, including lung carcinoma (Munson et al., 1975), thyroid epithelioma (Bifulco et al., 2001), lymphoma (McKallip et al., 2002), skin carcinoma (Casanova et al., 2003), and glioma (Galve-Roperh et al., 2000)—the focus of this mini-review.

Initial experiments in cultured glioma cells showed that incubation with cannabinoids induces cell death by an apoptotic mechanism (Sánchez et al., 1998). Further studies with animal models showed that local administration of THC or WIN-55,212-2 reduced the size of tumours generated by intracranial inoculation of C6 glioma cells in rats, leading to complete eradication of gliomas and subsequent survival in one-third of the treated rats (Galve-Roperh et al., 2000). Additional studies employed tumour xenografts generated by subcutaneous injection of glioma cells in the flank of immune-deficient mice. Local administration of THC, WIN-55,212-2 or the selective CB_2 agonist JWH-133 decreased the growth of tumours derived not only from the rat glioma C6 cell line, but also from GBM cells obtained from tumour biopsies of patients (Galve-Roperh et al., 2000; Sánchez et al., 2001a). Based on these and other observations, the Spanish Ministry of Health has approved a phase I/II clinical trial, performed in collaboration between the Tenerife University Hospital and our laboratory, aimed at investigating the effect of local administration of THC on the growth of recurrent GBM (Guzmán, 2003).

Although the downstream events by which cannabinoids exert their antiproliferative action in gliomas are

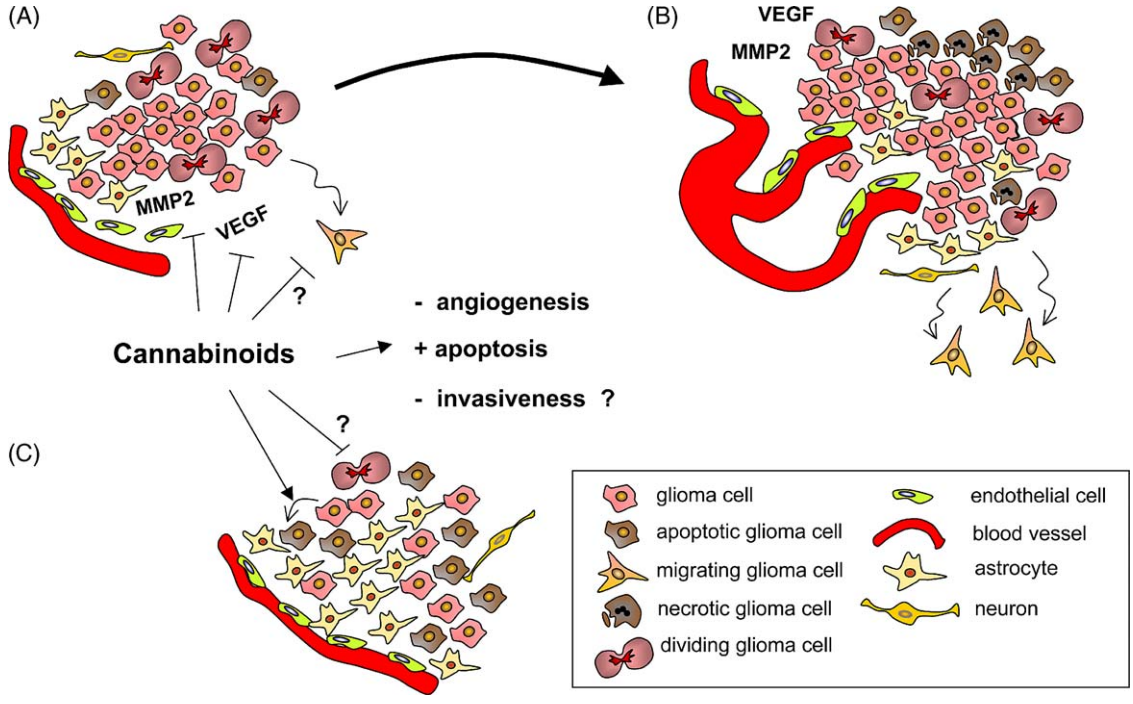


Fig. 2. Speculative model of cannabinoid antitumoural action in gliomas. (A) An early stage glioma; (B) The high proliferation rate of tumour cells leads to increased growth and therefore enhanced blood supply demand of gliomas. This leads in turn to the production of proangiogenic factors such as VEGF and of enzymes that allow tissue breakdown and remodelling such as MMP2; (C) Cannabinoids may mediate their antitumoural actions in gliomas via two mechanisms: induction of apoptosis—and perhaps growth arrest—of glioma cells, and inhibition of tumour angiogenesis. The latter effect could depend on (i) a direct action of cannabinoids on tumour cells by inhibiting proangiogenic factor production, and (ii) the inhibition of vascular endothelial cell migration and survival. In addition, cannabinoids might inhibit the expression of matrix metalloproteinases and other factors potentially involved in tumour cell invasiveness.

not completely unraveled, there is substantial evidence for the implication of at least two mechanisms: induction of apoptosis of tumour cells, and inhibition of tumour angiogenesis (Fig. 2).

3.1. Induction of apoptosis of tumour cells

Cultured glioma cells undergo apoptosis upon long-term cannabinoid challenge (Sánchez et al., 1998; Galve-Roperh et al., 2000). Activation of cannabinoid receptors and accumulation of the proapoptotic sphingolipid ceramide seem necessary for glioma cell apoptosis. Of interest, following cannabinoid receptor activation, two peaks of ceramide generation are observed in glioma cells that have different kinetics (minute- versus day-range), magnitude (two- versus four-fold), mechanistic origin (sphingomyelin hydrolysis versus de novo ceramide synthesis) and function (metabolic regulation versus induction of apoptosis) (Galve-Roperh et al., 2000; Guzmán et al., 2001a). Cannabinoids have been suggested to enhance ceramide synthesis de novo via induction of serine palmitoyltransferase, a regulatory enzyme of sphingolipid biosynthesis (Gómez del Pulgar et al., 2002a) (Fig. 3). It is worth noting that ceramide content has been inversely related with malignant progression and poor prognosis of human astrocytomas. Thus, low grade astrocytomas have higher ceramide content than high grade astrocytomas (Riboni et al., 2002). Moreover, it has been shown that low grade astrocytomas have lower CB_2 receptor expression than high grade astrocytomas (Sánchez et al., 2001a), suggesting that this particular cannabinoid receptor subtype may be a marker for brain tumour malignancy.

The increased ceramide levels observed in glioma cells upon cannabinoid challenge leads to prolonged activation of the Raf-1/MEK/ERK signalling cascade (Galve-Roperh et al., 2000). It is generally accepted that ERK activation leads to cell proliferation. However, the relation between ERK activation and cell fate is complex and depends on many factors, one of which is the duration of the stimulus as prolonged cannabinoid-induced ERK activation may mediate cell cycle arrest and cell death (Melck et al., 1999; Galve-Roperh et al., 2000). Sustained Akt inhibition (Gómez del Pulgar et al., 2002a) (Fig. 3) and c-Jun N-terminal kinase and p38 mitogen-activated protein kinase activation (Galve-Roperh et al., 2000) may also contribute to glioma cell death. Nevertheless, further investigation is necessary to clarify the specific downstream targets of these pathways involved in cannabinoid-induced apop-

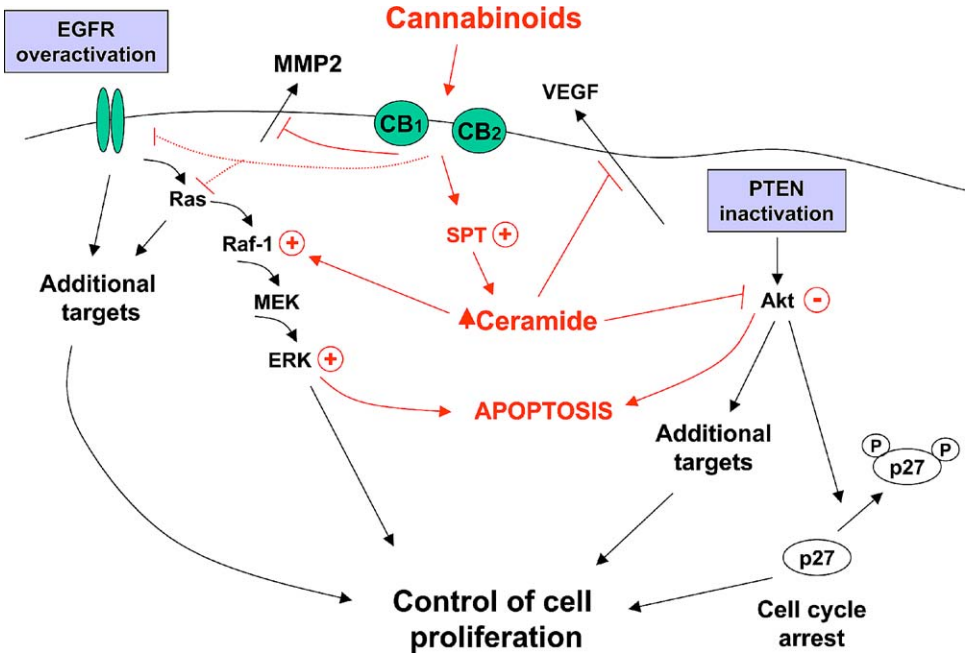


Fig. 3. Intracellular mechanisms of cannabinoid antitumoural action in gliomas. Inactivation of PTEN and overexpression of EGFR—or a constitutively active form of this receptor—are two of the most frequent alterations found in human gliomas. Tumours carrying these modifications would normally exhibit increased Akt activity, leading in turn to resistance to apoptosis and increased cell proliferation. Cannabinoid treatment may attenuate such alterations in glioma cells by inducing serine palmitoyltransferase (SPT) and promoting ceramide accumulation and Akt inhibition, which would decrease the phosphorylation of Akt and its downstream targets. In addition, ceramide induces the sustained stimulation of the Raf-1/MEK/ERK pathway, which has been shown to be involved in cannabinoid-induced apoptosis. Ceramide may also attenuate the production of the proangiogenic factor VEGF. Red lines: pathways affected by cannabinoids in glioma cells; dotted lines: other potential targets of cannabinoid antitumoural action in glioma cells; (+) activation; (−) inhibition.

tosis of glioma cells and the relative contribution of this apoptotic mechanism in vivo.

3.2. Inhibition of tumour angiogenesis

To grow beyond minimal size, tumours must generate a new vascular supply (angiogenesis) for purposes of cell nutrition, gas exchange and waste disposal, and therefore blocking the angiogenic process constitutes one of the most promising antitumoural approaches currently available (Kerbel and Folkman, 2002). Immunohistochemical and functional analyses in mouse models of glioma (Blázquez et al., 2003) and skin carcinoma (Casanova et al., 2003) have shown that cannabinoid administration turns the vascular hyperplasia characteristic of actively growing tumours to a pattern of blood vessels characterized by small, differentiated and impermeable capillaries. This is associated with a reduced expression of vascular endothelial growth factor (VEGF) and other proangiogenic cytokines (Casanova et al., 2003; Blázquez et al., 2003; Portella et al., 2003), as well as of VEGF receptors (Portella et al., 2003; Blázquez and Guzmán, unpublished results). Interestingly, pharmacological inhibition of ceramide synthesis de novo abrogates the antitumoural effect of cannabinoids in vivo as well as the cannabinoid-induced inhibition of VEGF production by glioma cells in vitro and by gliomas in vivo (Blázquez and Guzmán, unpublished results), indicating that ceramide may play a general role in cannabinoid antitumoural action. In addition, activation of cannabinoid receptors in vascular endothelial cells inhibits cell migration and survival, which might contribute as well to the impaired tumour vascularization observed in cannabinoid-treated gliomas (Blázquez et al., 2003) (Fig. 2).

Cannabinoids have been recently shown to reduce the number of metastatic nodes produced from paw injection of Lewis lung carcinoma cells in rats (Portella et al., 2003). Moreover, cannabinoid administration to glioma-bearing mice decreases the activity and expression of matrix metalloproteinase-2 (MMP2), a proteolytic enzyme that allows tissue breakdown and remodelling during angiogenesis and metastasis (Blázquez et al., 2003). Hence, it is conceivable that cannabinoids may also control glioma invasiveness (Fig. 2).

3.3. Other potential targets of cannabinoid action

The findings discussed above indicate that cannabinoid administration regulates an array of processes involved in the induction of apoptosis, inhibition of angiogenesis and, perhaps, reduction of invasiveness in gliomas (Fig. 2). However, though mainly based on indirect observations, several reports suggest that the antitumoural action of cannabinoids in gliomas may also be due to inhibition of glioma cell growth and proliferation. Thus, cannabinoids have been shown to induce cell cycle arrest in breast carcinoma (De Petrocellis et al., 1998), prostate carcinoma (Melck et al., 2000) and thyroid epithelioma cells (Bifulco et al., 2001). In the latter cells, this effect has been attributed to a cannabinoid-triggered induction of the cyclin-dependent kinase inhibitor $p27^{kip1}$ (Portella et al., 2003). Because cannabinoid treatment decreases Akt activity in glioma cells (Gómez del Pulgar et al., 2002a), and phosphorylation by Akt blocks the inhibitory activity of $p27^{kip1}$ by favouring its cytoplasmic localization (Viglietto et al., 2002), it is conceivable that this mechanism could operate in cannabinoid-treated gliomas. Noteworthy, cannabinoid administration to glioma-bearing nude mice leads to growth inhibition rather than to an actual reduction of tumour size (Galve-Roperh et al., 2000; Sánchez et al., 2001a).

Cannabinoids have been suggested to mediate at least part of their growth-inhibiting actions on skin and prostate cancer cells by attenuating epidermal growth factor receptor (EGFR) tyrosine kinase activity (Casanova et al., 2003) and/or by lowering EGFR expression (Casanova et al., 2003; Mimeault et al., 2003). Furthermore, the antiproliferative action of cannabinoids in breast, prostate and thyroid cancer cells might involve a decrease in the activity and/or expression of prolactin (De Petrocellis et al., 1998), nerve growth factor (Melck et al., 2000) or VEGF (type 1) tyrosine kinase receptors (Portella et al., 2003). In addition, cannabinoids inhibit VEGF (type 2) receptor in glioma cells (Blázquez and Guzmán, unpublished results). Taken together, these results indicate that attenuation of the signalling through tyrosine kinase receptors may constitute a common mechanism of cannabinoid antiproliferative action. However, it has been recently shown that THC induces proliferation as well as ERK and Akt activation in glioma cells via EGFR transactivation (Hart et al., 2004). In line with previous findings on glioma cell death/survival (Galve-Roperh et al., 2002) and the duality of some other cannabinoid effects on cell fate (Guzmán et al., 2001b), that observation might be attributed to the use of low cannabinoid concentrations (nanomolar range) and short times of cannabinoid exposure (18 h) (Hart et al., 2004). It is therefore conceivable that cannabinoids could favour (at low signal input) or inhibit (at high signal input) tumour cell growth. In fact, Hart et al. (2004) also found that micromolar concentrations of THC-induced apoptosis of glioma cells.

Although the identification of the cell(s) of origin of gliomas is still a matter of debate, it has been shown that transformed neural progenitor cells can give rise to these tumours (Holland et al., 2000; Reilly et al., 2000). In addition, dedifferentiation of astrocytes to glial

progenitor-like cells leads to the generation of gliomas (Dai et al., 2001). Interestingly, cannabinoids may play a role in the control of neural progenitor cell differentiation and proliferation (Rueda et al., 2002), suggesting that cannabinoids might also influence gliomagenesis by altering neural progenitor cell fate.

4. Cannabinoids as potential therapeutic agents for gliomas

4.1. Selectivity of antiproliferative action

Although it has been reported that THC may have deletereous effects on primary neurons in culture (Chan et al., 1998; Downer et al., 2003), most of the experimental evidence supports that cannabinoids are capable of killing transformed neural cells selectively (Guzmán, 2003). Thus, in contrast with their proapoptotic and antitumoural effect on gliomas, cannabinoids protect normal astroglial (Gómez del Pulgar et al., 2002b) and oligodendroglial (Molina-Holgado et al., 2002) cells from apoptosis. This protective effect is mediated by the CB_1 receptor and the PI3K/Akt survival pathway, with a possible additional contribution of ERK. As discussed above, cannabinoids induce apoptosis of glioma cells via ceramide generation (Galve-Roperh et al., 2000; Gómez del Pulgar et al., 2002a), whereas they attenuate apoptosis of normal astrocytes as induced by ceramide (Gómez del Pulgar et al., 2002b) and oxidative stress (Carracedo et al., 2004).

The molecular basis of this "ying-yang" behaviour is not completely understood yet, but could result from the differential capacity of tumour and non-tumour cells to synthesise ceramide in response to cannabinoids. Thus, cannabinoid receptor activation in glioma cells enhances ceramide synthesis de novo and triggers apoptosis, whereas in normal astrocytes ceramide synthesis de novo or apoptosis are not induced despite the presence of functional cannabinoid receptors (Guzmán et al., 2001a; Guzmán, 2003). Accordingly, cannabinoids may inhibit Akt via ceramide in glioma cells (Gómez del Pulgar et al., 2002a), while in primary astrocytes they activate Akt and abrogate ceramide-induced Akt inhibition (Gómez del Pulgar et al., 2002b). It would be expected that cannabinoid receptors are downregulated when apoptosis of cultured glioma cells occurs upon long-term cannabinoid challenge, suggesting that the apoptotic response of glioma cells has been already triggered at that point. Intriguingly, it has been reported that prolonged stimulation of thyroid epithelioma cells with cannabinoids leads to overexpression of the CB_1 receptor (Bifulco et al., 2001; Portella et al., 2003). Thus, the opposite response to cannabinoids of transformed and non-transformed glial cells may rely on differential features of their respective intracellular signalling pathways and/or on differences in the functionality of their cannabinoid receptors.

4.2. Cannabinoid side-effects

Cannabinoids have a favourable drug safety profile. Acute fatal cases due to cannabis use in humans have not been substantiated, and median lethal doses of THC in animals have been extrapolated to several grams per kilogram body weight (Grotenhermen, 2003). The use of cannabinoids in medicine, however, is severely limited by their psychoactive effects. Although these adverse effects are within the range of those accepted for other medications in cancer treatment, and tend to disappear with tolerance upon continuous use, it is obvious that cannabinoid-based therapies devoid of side-effects would be desirable. As the unwanted psychotropic effects of cannabinoids are mediated largely or entirely by CB_1 receptors within the brain, a first possibility would be to use cannabinoids that target CB_2 receptors. Selective CB_2 receptor activation in mice induces regression of gliomas (Sánchez et al., 2001a) and skin carcinomas (Casanova et al., 2003) in mice without overt signs of psychoactivity. One must be, however, cautious in this respect, as not all human GBMs and glioma cell lines express the CB_2 receptor (Sánchez et al., 2001a; Cudaback et al., 2003). A better understanding of the factors that control the expression of this receptor would be very helpful to support the use of selective CB_2 agonists in the management of GBM. It is also worth noting that CB_2 agonists might impair host antitumour immunity (Zhu et al., 2000).

Cannabinoids that act via putative non-CB receptors and are therefore devoid of psychoactivity might also be useful in glioma therapy. These include cannabidiol (Jacobsson et al., 2000; Massi et al., 2004) and ajulemic acid (Recht et al., 2001), both of which inhibit the growth of glioma cells in culture and in mice. In addition, TRPV1 vaniloid receptors could participate in cannabinoid-induced apoptosis of glioma and neuroblastoma cells (Maccarrone et al., 2000; Jacobsson et al., 2001).

4.3. Towards a more selective therapy

GBM cells display a high morphological and behavioural variability even within the same tumour (Maher et al., 2001). During the last few years, investigators have started to learn about the varying alterations associated with the different stages of gliomas, including GBM (Maher et al., 2001; Merlo, 2003; Mischel et al., 2003). For example, inactivation of the tumour-suppressor phosphatase PTEN, which leads to stimulation of the PI3K/Akt cascade, is present in

30–40% of high-grade gliomas (Maher et al., 2001). Because cannabinoids inhibit Akt in glioma cells (Gómez del Pulgar et al., 2002a), it is conceivable that their antitumoural action may be relevant in cells carrying PTEN mutations (Fig. 3). Likewise, a high percentage of gliomas overexpress EGFR or a truncated constitutively active form of this receptor (Maher et al., 2001). As cannabinoids blunt EGFR signalling in skin (Casanova et al., 2003) and prostate (Mimeault et al., 2003) cancer cells, their administration might curb the growth of glioma cells carrying alterations in this receptor (Fig. 3).

In order to improve the efficiency of cannabinoid utilization in glioma therapy it would be desirable to correlate the modifications accumulated in each type of glioma with their susceptibility to cannabinoid treatment. A preliminary study in this direction indicates that cannabinoid-induced apoptosis of three human astrocytoma cell lines (U373MG, T98G and U87MG) in culture is independent of their p53 status (Kaminska, personal communication). Cannabinoids should also be tested in combination with other chemotherapeutic drugs or radiotherapy to establish whether they can enhance current drug treatments. Interestingly, compounds that induce cell death via ceramide have proved useful in combined therapies (Radin, 2003).

In conclusion, the significant antiproliferative action of cannabinoids in animal models of gliomas, together with their low toxicity compared with other chemotherapeutic agents, might make these compounds promising new tools for the management of GBM. However, one must be extremely cautious in this respect. Medicine has been unsuccessful for several decades in striving against GBM, and today there is little doubt that it will be necessary to use selective strategies, including most likely the combination of several drugs and therapeutic approaches, to achieve a significant improvement in the treatment of this devastating disease.

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