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MINI REVIEW

Cannabinoids and cancer: pros and cons of an antitumour strategy

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In the last two decades, research has dramatically increased the knowledge of cannabinoids biology and pharmacology. In mammals, compounds with properties similar to active components of *Cannabis sativa*, the so called 'endocannabinoids', have been shown to modulate key cell-signalling pathways involved in cancer cell growth, invasion and metastasis. To date, cannabinoids have been licensed for clinical use as palliative treatment of chemotherapy, but increased evidences showed direct antiproliferative actions of cannabinoid agonists on several tumour cells *in vitro* and in animal models. In this article, we will review the principal molecular pathways modulated by cannabinoids on cancer and summarize *pros* and *cons* evidence on the possible future use of endocannabinoid-based drugs in cancer therapy.
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Introduction

The endocannabinoid system, that is, the cannabinoid receptors, endogenous cannabinoid ligands and endocannabinoid-metabolizing enzymes, has drawn a great deal of scientist attention during the past 15 years. The use of cannabinoids in the treatment of cancer chemotherapy side effects was the most studied potential therapeutic application. Powerful chemotherapy side effects can be very severe and intolerable: reported beneficial effects from cannabinoids use, in chemotherapy patients, are a reduced incidence and severity of emesis, appetite stimulation, improvement of cachexia and pain inhibition. Marijuana's major active principle, Δ[9]-tetrahydrocannabinol (THC), has been licensed for clinical use as palliative treatment for cancer patients, in two preparations, dronabinol and its analogue nabilone. Moreover, mammals produce at least two endogenous compounds anandamide (AEA, *N*-arachidonoyl-ethanolamine) and 2-arachidonoylglycerol (2-AG) selectively acting on the same receptors as THC.

The 'endocannabinoid' system seems to be involved in an increasing number of diseases and to hold promise for development of new therapeutic drugs without psychoactive effects peculiar to THC. Increasing evidence showed a direct antitumour activity of cannabinoid agonists in a plethora of tumour cells including breast, brain, skin, thyroid, prostate and colorectal. This effect was due to the inhibition of tumour growth mediated by cell-cycle arrest or apoptosis, as well as reduction in neovascularization and metastases. When these findings will be supported by *in vivo* studies, beside their therapeutical implication, they might open new insight on endogenous mechanisms of tumour suppression.

The endocannabinoid system

The discovery of a family of endogenous cannabinoids, named endocannabinoids (Devane *et al.*, 1992; Sugiura *et al.*, 1995), have focused much attention on cannabinoids during the past years. Two different cannabinoid receptors have been cloned from mammalian tissues: cannabinoid receptor 1 (CB1), originally named 'central' receptor (Matsuda *et al.*, 1990) and CB2, also incorrectly known as 'peripheral' receptor (Munro *et al.*, 1993), and an increasing number of reports and pharmacological evidence suggest that endocannabinoids might also exert their biological effects through non-CB1/CB2 receptors (Di Marzo *et al.*, 2000; Kunos *et al.*, 2000; Maccarrone *et al.*, 2000).

Both the CB1 and CB2 genes encode a seven-transmembrane-domain protein belonging to the Gαi protein-coupled

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receptor family (Munro *et al.*, 1993): the signal transduction pathway downstream cannabinoid receptors includes adenylate cyclase (Howlett *et al.*, 1986), mitogen-activated protein kinase (MAPK) (Bouaboula *et al.*, 1995) and, in the case of CB1, ion channels (Mackie & Hille, 1992). Whereas CB1 is preferentially expressed in the central nervous system (Matsuda *et al.*, 1990), CB2 has been described as the predominant form expressed in peripheral immune cells (Munro *et al.*, 1993; Galiegue *et al.*, 1995).

The central and most of the peripheral effects of cannabinoids rely on CB1 activation. This receptor is detectable in several brain areas, at very high levels in the basal ganglia, hippocampus, cerebellum and cortex, where it mediates cannabinoid psychoactive effects; its expression during brain development is significantly different from the one observed in the adult stage (Berrendero *et al.*, 1999). CB1 receptors are also present in peripheral nerve terminals, as well as in extra-neural tissues such as testis, uterus, vascular endothelium, eye, spleen, ileum and in adipocytes (Matsuda *et al.*, 1990; Munro *et al.*, 1993; Felder & Glass, 1998; Straiker *et al.*, 1999; Liu *et al.*, 2000; Pertwee, 2000; Cota *et al.*, 2003). The CB2 receptor is believed to be expressed in immune cells and it is unrelated to cannabinoid psychoactive effects (Felder & Glass, 1998). The CB2 is normally expressed in areas enriched of B lymphocytes such as the spleen marginal zone, the lymph node cortex, the nodular corona of Peyer patches and the mantle zones of secondary follicles in tonsils (Munro *et al.*, 1993; Lynn & Herkenham, 1994; Galiegue *et al.*, 1995; Howlett *et al.*, 2002). CB2 receptors were found in microglia cells (Kearn & Hillard, 1997; Walter *et al.*, 2003; Nunez *et al.*, 2004), in glioma and in skin tumour cells (Casanova *et al.*, 2001; Di Marzo *et al.*, 2004). The CB2 receptor is involved in B-cell differentiation and migration of splenic B lymphocytes, suggesting a role for this receptor in the immune response (Galiegue *et al.*, 1995; Carayon *et al.*, 1998). A recent study (Jorda *et al.*, 2004) showed that CB2 was overexpressed in several human myeloid leukaemia cell lines; interestingly, in retrovirus-induced myeloid leukaemia models, the *Cb2* gene was located in a common virus integration site, EVI1, suggesting that *Cb2* could be a proto-oncogene involved in transformation (Valk *et al.*, 1997). Endogenous ligands for the cannabinoid receptors are lipid molecules containing long-chain polynsatured fatty acids,

amides, esters and ethers, with different selectivity for the two receptor types (Mcallister & Glass, 2002; Mechoulam *et al.*, 2002). The best-known endogenous cannabimimetics are AEA (also called Anandamide) and another arachidonate derivative, 2-AG (Devane *et al.*, 1992; Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). Moreover, *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine (OEA) and *N*-stearoylethanolamine (SEA) compounds called 'endocannabinoid-like' are present in human, rat and mouse brain (Di Marzo, 1998; Maccarrone & Finazzi-Agrò, 2002) where they might inhibit the degradation of AEA or 2-AG and, consequently, increase their activity (Mechoulam *et al.*, 2002). In the central nervous system, endocannabinoids act as neuromodulators or retrograde messengers (MacDonald & Vaughan, 2001) which inhibit the release of various neurotransmitters (Schlicker & Kathmann, 2001); in the peripheral and neural tissues, they modulated the effects of proteins and nuclear factors involved in cell proliferation, differentiation and apoptosis, as paracrine or autocrine mediators. These data suggested that endocannabinoids could play a role in the control of cell fate (Guzman *et al.*, 2001b).

The most exciting studies reported the potential use of cannabinoids as therapeutic agents (Piomelli *et al.*, 2000; Porter & Felder, 2001). It is now unquestionable that cannabinoids are effective as antiemetic agents in vomiting induced by anticancer drugs (Joy *et al.*, 1999) and increasing evidence suggests the efficacy of cannabinoids for treatment of various diseases such as glaucoma, multiple sclerosis, brain injuries, cardiovascular disorders, chronic inflammation diseases (Mechoulam *et al.*, 2002; Baker *et al.*, 2003; Guzman, 2003; Mendizabal & Adler-Graschinsky, 2003; Kunos & Pacher, 2004; Tomida *et al.*, 2004). Hopes for these possible applications encouraged the development of new synthetic cannabinoid-related drugs capable of a more selective activation of cannabinoid receptors. Principal compounds and their actions are summarized in Table 1. To date, these substances have been extensively used, both *in vitro* and *in vivo*, as pharmacological tools to obtain more detailed insight of cannabinoid action, in order to evaluate their potential clinical use. There is mixed evidence on the effects of cannabinoids on cancer: *in vitro* and *in vivo* studies and clinical data showed both antineoplastic and protumoral activity, depending on

Table 1 Properties of cannabinoid-related drugs

Compound	Target(s)	Potential therapeutic applications
CP-55,940	Nonselective agonist (CB1 = CB2)	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor, multiple sclerosis
WIN 55,212-2	Nonselective agonist (CB1 = CB2)	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor, multiple sclerosis
HU-210	Nonselective agonist (CB1 = CB2)	Analgesic, multiple sclerosis, neuroprotective
Δ^9-THC	Nonselective agonist (CB1 > CB2)	Analgesic, antiemetic, appetite stimulant tumour growth inhibitor
Anandamide	Nonselective agonist (CB1 ≫ CB2)	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor
(R)-methanandamide	Nonselective agonist (CB1 ≫ CB2) metabolically stable	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor
2-AG	Nonselective agonist (CB1 > CB2)	Analgesic, antiemetic, appetite stimulant, tumour growth inhibitor
O-1269	Partial CB1 agonist	
Noladin ether	Selective CB1 agonist	Analgesic
AM-1241	Selective CB2 agonists	Tumour growth inhibitor (in glioma, skin carcinoma, lymphoma and leukaemia); multiple sclerosis immune diseases peripheral analgesia
HU-308		
JWH-133		
JWH-015		
BML190	Nonselective agonist (CB2 ≫ CB1)	

type of agonist, target tissues, route of administration, doses and duration of the treatment.

In this article, we will review the principal molecular pathways modulated by cannabinoids in cancer cells and summarize pros and cons evidence for a possible use of cannabinoid-based drugs in cancer therapy in the future.

Cannabis smoke intake and cancer

Several studies produced exciting new leads in the search for anticancer treatments using cannabinoid-related drugs. Plant-derived (THC), synthetic (HU210, WIN-55,212-2), and endogenous (2-AG, AEA) cannabinoids modulate tumour growth, apoptosis, migration and neoangiogenesis in various types of cancer (Bifulco & Di Marzo, 2002; Guzman *et al.*, 2002). However, studies performed to investigate marijuana-smoking effects on carcinogenesis and tumour growth produced contradictory results (Table 2): THC failed to induce mutagenicity in the Ames test (Hall & MacPhee, 2002) and in skin test in mice (Chan *et al.*, 1996), whereas cannabis smoke was mutagenic *in vitro* (MacPhee, 1999; Marselos & Karamanakos, 1999). The Ames test is a sensitive biological method for measuring the potentially carcinogenic effect of chemical substances on microorganisms, cells and tissue cultures. This test by itself does not demonstrate cancer risk; however, mutagenic potency evaluated by Ames test does correlate with the carcinogenic potency for chemicals in rodents. These results show that THC have no carcinogenic properties, at least as purified compound. Moreover, evidence showed that smoking of cannabis preparations caused cancer of the respiratory and oral tracts or, at least, potentiated tobacco smoke-induced damages. Various mechanisms have been involved in these processes: direct THC-induced damage of the bronchial epithelium (Barsky *et al.*, 1998), induction and regulation of the carcinogen-metabolizing enzyme CYP1A1 (Roth *et al.*, 2001), alteration of the balance between apoptotic and necrotic cell death (Sarafian *et al.*, 2001), increase of cellular oxidative stress (Sarafian *et al.*, 1999), CB2-mediated immune suppression (Srivastava *et al.*, 1998; Zhu *et al.*, 2000). Recently, Hall *et al.* (2005) extensively reviewed the results of epidemiological studies reporting inconsistent association between cannabis smoking and lung cancer. The author highlighted the need of a case–control cohort larger than those previously examined, excluding concomitant risk factors as alcohol use or tobacco smoke. Furthermore, the cannabis smoking and the medical use of cannabinoids have been largely mistaken in public debate: the recreational long-term cannabis smoking, potentially but to date ambiguously connected with respiratory and oral cancer, is not univocally associated with pharmaceutical cannabinoids exploitable for medical purposes.

Effects of cannabinoids on tumour biology: modulation of key cell-signalling pathways involved in control of cell fate

Breast and prostate cancer and cannabinoids

Studies performed in order to understand the role of endocannabinoids and their receptors in the control of cell fate raised great interest (Table 2). In 1998, De Petrocellis *et al.* investigated the possible antimitogenic effects of AEA on epithelial human breast cancer cell (HBCC) lines EFM-19 and MCF-7, expressing oestrogen and prolactin receptors and proliferating in response to steroid or lactogenic hormones treatments (Simon *et al.*, 1985; Clevenger *et al.*, 1995). In these models, treatment with submicromolar concentration of AEA (as well as of 2-AG or HU-210) significantly inhibited the G1–S transition of mitotic cell cycle. Moreover, anandamide inhibited the expression of prolactin receptor, induced down-regulation of the *brca1* gene product (De Petrocellis *et al.*, 1998), and of *trk* proteins, the high-affinity neurotrophin receptors (Melck *et al.*, 1999b, 2000). The antiproliferative CB1-receptor-mediated effect was AEA dose-dependent and proportional to the degree of hormone dependency of the used HBCC line (De Petrocellis *et al.*, 1998). The block of the G1–S transition was ascribed to the inhibition of adenylyl cyclase and, consequently of cAMP-protein kinase A pathway and to the activation of MAPK (Melck *et al.*, 1999b). Cannabinoids prevented the inhibition of RAF1 (caused by protein kinase A-induced Raf phosphorylation) and induced prolonged activation of the RAF1-MEK-ERK signalling cascade, leading to downregulation of PRLr and Trk (Melck *et al.*, 2000).

On the other hand, a recent report (McKallip *et al.*, 2005) demonstrated that HBCC lines MCF-7 and MDA-MB-231, and the mouse mammary carcinoma 4T1, are resistant to THC-induced cytotoxicity. The authors hypothesized that the degree of tumour sensitivity to THC may be related to the level of CB1 and CB2 expression, and that THC exposure may lead to an increase in growth rate and metastatic potential of tumours with low to no expression of cannabinoid receptors. It is an unsurprising data that different clones of the same cell lines, as well as of breast cancer cells, showed very variable levels of receptors and a different responsivity to hormone and growth factors (Hamelers *et al.*, 2003). Cannabinoid receptors expression could be at least in part modulated by the culture conditions and the number of subculturing passages, even in the absence of specific ligands (Melck *et al.*, 2000). In addition, McKallip *et al.* specify that 4T1 cells expressed high levels of vanilloid receptor (VR1), a nonselective cation channel, activated by capsaicin, which is also a characterized target for AEA. This observation could be very interesting because these breast cancer cells may be more sensitive to AEA (Melck *et al.*, 1999a; Smart *et al.*, 2000; Zygmunt *et al.*, 2000), rather than to THC.

Proliferative disorders of the prostatic gland involve multi-step process and sequential changes in the responsiveness of prostate epithelial cells to steroid hormones, growth factors and neuropeptides (Marker *et al.*, 2003). Several intraepithelial or invasive prostatic cancers showed increased expression of epidermal growth factor receptor (EGF-R) tyrosine kinase, EGF and transforming growth factor α (TGFα) (Liu *et al.*, 1993; Ware, 1993; Kim *et al.*, 1999). Moreover, androgen-independent human prostate cancer cell lines PC3 and DU145 overexpressed EGF-R, which, *via* a selective interaction with autocrine and paracrine-secreted EGF and TGFα, promoted cell proliferation. In these models, androgen and EGF downregulated p27^{kip}, an inhibitor of cyclin-dependent protein kinases (Peng *et al.*, 1996; Wu *et al.*, 1996; Ye *et al.*, 1999). Mimeault *et al.* (2003) showed that a micromolar concentration of AEA inhibited EGF-induced proliferation of DU145 and PC3 cells, as well as of androgen-stimulated LNCaP, *via*

Table 2 Potential use of cannabinoids in cancer treatment: pro and cons evidence

Tumour (cell type)	Cannabinoid (concentration or dose)	Anticancer effect	Procancer effect	Mechanism of action	References
Bronchial epithelium	THC		+	Molecular abnormalities and histopatological alterations	Barsky et al. (1998)
Murine hepatoma cell line (Hepa)	THC (2–10 μg/ml)		+	Induction of CYP1A1	Roth et al. (2001)
Lung cancer cell line (A549)	THC		+	Inhibition of Fas-induced caspase-3 activity	Sarafian et al. (2001)
Endothelial cell line	THC (1.77 or 3.95%)		+	Increased ROS generation	Sarafian et al. (1999)
Murine Lewis lung carcinoma (3LL); alveolar cell carcinoma (L1C2)	THC (5–40 mg/kg)		+	In vivo, decreased production of cytokines and/or CB2-mediated immune suppression	Zhu et al. (2000)
	CBD (⩾5 μg/ml)		+		Srivastava et al. (1998)
Human breast cancer cell lines (MCF7; EFM-19)	AEA (2–10 μM) / 2-AG (2–10 μM) / HU210 (⩾4 μM)	+		Inhibition of the mitogen-induced stimulation of the G0/G1–S phase	De Petrocellis et al. (1998)
	AEA (⩾2 μM) / 2-AG, HU210 (⩾1 μM)	+			Melck et al. (2000)
Human breast cancer cell lines (MCF7; MDA-MB-231) Mouse mammary carcinoma (4T1)	THC (⩽5 μM)		+	Increased tumour growth and metastasis; in vivo, decreased antitumour immune response	McKallip et al. (2005)
Androgen-independent prostate cancer cells (PC3, DU145)	AEA, R-(+)-MET (⩾2 μM)	+		Inhibition of mitogen-induced proliferation, G1 arrest	Mimeault et al. (2003) Melck et al. (2000)
	THC (1 μM)	+		Apoptosis	Ruiz et al. (1999)
Androgen-dependent prostate cancer cells (LNCaP)	AEA, R-(+)-MET (⩾2 μM)	+		Inhibition of mitogen-induced proliferation, G1 arrest	Mimeault et al. (2003)
Androgen-dependent prostate cancer cells (LNCaP)	WIN-55,212-2 (⩾2.5 μM)	+		Dose- and time-dependent induction of apoptosis; decreased expression of AR and PSA	Sarfaraz et al. (2005)
Androgen-dependent prostate cancer cells (LNCaP)	R-(+)-MET (0.1–0.2 μM)		+	Increased proliferation and AR expression	Sanchez et al. (2003)
Rat glioma cell line (C6)	THC (1 μM)	+		Apoptosis via ceramide de novo synthesis In vivo, regression of C6-derived glioma	Galve-Roperh et al. (2000)
	JWH133, WIN-55,212-2 (0.1 μM)	+		Apoptosis via ceramide de novo synthesis	Sanchez et al. (2001a, b)
	WIN-55,212-2 (15 μM)	+		Apoptosis via activation of caspase cascade	Ellert-Miklaszewska et al. (2005)
Human astrocitoma (grade IV)	JWH-133 (50 μg/die)	+		In vivo, inhibited growth of tumours induced in deficient mice	Sanchez et al. (2001a, b)
Human glioblastoma multiforme cell line (GBM)	THC (1 μM) / WIN-55,212-2	+		Decreased proliferation and increased cell death	McAllister et al. (2005)
K-ras-transformed FRTL-5 thyroid cells (KiMol)	Met-F-AEA (0.5 ng/kg/dose)	+		In vivo, inhibited growth of tumours induced in nude mice	Bifulco et al. (2001)
Mouse skin carcinoma cells (PDV-C57)	JWH-133, WIN-55,212-2 (1.58 μg)	+		In vivo, inhibited growth of tumours induced in nude mice	Casanova et al. (2003)
Human umbilical vein endothelial cells (HUVEC)	JWH-133 (25 nM)	+		Induction of apoptosis, inhibited migration	Blazquez et al. (2003)
Lung cancer cells (NCI-H292) Glioblastoma cell line (U373-MG)	THC (0.1–0.3 μM)		+	Increased proliferation	Hart et al. (2004)
Human breast cancer cell line (MDA-MB-231) Mouse breast cancer cell line (TSA-E1)	Met-F-AEA (10 μM and 0.5 mg/kg/dose)	+		Inhibition of adhesion and migration In vivo, reduction of number and dimension of metastatic nodes	Grimaldi et al. (2006)

G1 arrest, and downregulated EGF-R levels. Both phenomena were CB1-mediated. Similar growth arrest and receptor modulation were also reported for prolactin- and nerve growth factor-stimulated DU145 (De Petrocellis *et al*., 1998; Melck *et al*., 2000). It is important to remark that longer AEA-incubation times (5–6 days) were able to induce massive apoptosis in DU145 and PC3 cells. This effect was mediated by CB1/2 *via* cellular ceramide accumulation, and was absent in LNCaP cells (Mimeault *et al*., 2003). Furthermore, micromolar WIN-55,212-2 treatment significantly decreased LNCaP cells viability and androgen receptor (AR) expression in a dose- and time-dependent manner, with maximal effect at 72 h (Sarfaraz *et al*., 2005). The authors described also a decrease in intracellular as well as in secreted levels of prostatic-specific antigen (PSA), an androgen-receptor-regulated glycoprotein (Montgomery *et al*., 1992) that currently is the most-accepted marker for assessment of prostate cancer progression (Stamey *et al*., 1987). Their results showed that treatment of LNCaP cells with WIN-55,212-2 also inhibited vascular endothelial growth factor (VEGF) protein expression, an ubiquitous cytokine with a key role in angiogenesis (Blazquez *et al*., 2003). Dose- and time-dependent effects of cannabinoids are a crucial issue to debate. It is puzzling that a 4-day treatment with *R*-(+)-methanandamide (MET) or exogenous cannabinoids, at submicromolar concentrations, increased the proliferation rate of LNCaP cells and the expression of AR, whereas longer incubation periods led to differentiation (Sanchez *et al*., 2003). Apparently, MET-induced mitogenic effect was phospho-kinase C (PKC)- rather than cAMP-pathway dependent; furthermore, in this cellular model, the androgen receptor expression was CB1- and, partially, CB2-mediated (Sanchez *et al*., 2003; Sarfaraz *et al*., 2005).

Depending on drug concentration, cannabinoids may either inhibit or stimulate cancer cell proliferation. Hart *et al*. (2004) found that treatment of several cancer cell lines with nanomolar concentration of THC, AEA, HU-210 or WIN-55,212-2 induced increased proliferation that was dependent on EGF-R phosphorylation. These data suggested that in a variety of human cancer cell lines CB1/CB2 receptors are linked to MAPK and AKT/PKB activation, and that cannabinoid concentrations could have dramatic effects in the cellular choice between proliferation and cell growth arrest.

Glioma and cannabinoids

The antitumoral action of cannabinoids on glioma may be exerted either *via* the CB1 or the CB2 receptor. THC induced apoptosis of C6 glioma cells by a pathway involving CB1 receptor, sustained generation of the proapoptotic lipid ceramide and prolonged activation of Raf1/MEK/ERK cascade (Galve-Roperh *et al*., 2000). A role for BCL-2 family members, such as Bad, have also been hypothesized (Ellert-Miklaszewska *et al*., 2005). Galve-Roperh *et al*. (2000) showed that cannabinoids induced regression of gliomas *in vivo*. In their model, intratumour administration of THC and WIN-55,212-2 induced regression of C6-derived glioma in Wistar rats and in RAG-2-deficient mice. In this study, they showed that cannabinoid administration induced no substantial modification in behavioural parameters, in food and water intake or in body weight; neurotoxicity nor markers of tissue damage have been revealed for at least 2 months after

cannabinoid treatment. Moreover, selective CB2 agonists showed good *in vivo* efficacy on regression of highly malignant human astrocitoma (grade IV) (Sanchez *et al*., 2001a). Ramer *et al*. (2003) demonstrated that cannabinoids induced the expression of cyclooxygenase-2 (COX-2) in human neuroglioma cells *via* a cannabinoid-receptors independent pathway, probably linked to lipid raft microdomains (Hinz *et al*., 2004). Since COX-2 can inhibit apoptosis (Tsujii & Dubois, 1995), these findings could demolish the promising effects of potential cannabinoids use in human gliomas, but additional studies showed that COX-2 induction may sensitize cells to apoptotic death (Corasaniti *et al*., 2000; Na & Surh, 2002) or rather finely regulate the cell choice between proliferation and death (Ramer *et al*., 2003). Cannabinoid receptors could have a protective role against programmed cell death, as reported in human neuroblastoma and C6 cells, where AEA induced apoptosis, *via* vanilloid receptors, increasing intracellular calcium concentration, activating COX, releasing cytochrome *c* and activating caspase 3 (Maccarrone *et al*., 2000). The mechanism through which AEA induces apoptosis in cells expressing both functional cannabinoid and vanilloid receptors is still controversial and might depend on the experimental conditions used. In fact, Jacobsson *et al*. (2001) showed that in rat glioma C6 cells, the AEA antiproliferative effect was associated with a combined activation of cannabinoid and vanilloid receptors and it was difficult to exclude a cannabinoid receptor role in the AEA-induced apoptotic cell death.

Cannabinoid receptor expression and endocannabinoid levels in transformed *versus* normal cells

Cannabinoid receptor levels seem to be a fundamental element for growth inhibitory effects. It has been documented that the expression of CB1 receptor was regulated in an opposite way in normal or transformed cells. Bifulco *et al*. (2001) demonstrated that met-fluoro-anandamide (Met-F-AEA) increased the levels of CB1 receptors in both K-ras-transformed FRTL-5 (KiMol) cells and in KiMol-derived tumours in nude mice, whereas in FRTL-5 cells, a thyroid-differentiated epithelial cell line, Met-F-AEA produced downregulation of CB1 receptors. Furthermore, cannabimimetic substances inhibited the proliferation of KiMol cells more strongly than of FRTL-5 cells; *in vivo*, Met-F-AEA inhibited growth of KiMol-induced tumours in athymic mice. These effects were accompanied by reduction of p21[ras] activity.

Apparently, an opposite regulation of CB1 expression in transformed *versus* normal cells was a common mechanism: THC induced apoptosis in several human cancer cell lines but showed less efficacy in nontransformed cell counterparts (Sanchez *et al*., 1998; Ruiz *et al*., 1999; Galve-Roperh *et al*., 2000; Guzman *et al*., 2001a; McAllister *et al*., 2005). Finally, cannabinoids protected oligodendroglial cells from various proapoptotic stimuli (Molina-Holgado *et al*., 2002) and astrocytes from ceramide-induced sensitization to oxidative damage (Carracedo *et al*., 2004), whereas they induced apoptosis of glioma cells (Galve-Roperh *et al*., 2000; Sanchez *et al*., 2001b; Gomez del Pulgar *et al*., 2002). A recent study showed a different endocannabinoid metabolism in human glioblastoma and meningiomas (Petersen *et al*., 2005): glioblastoma were characterized by increased levels of AEA and decreased fatty acid amide hydrolase (FAAH) activity, while meningiomas showed enhanced levels of 2-AG compared

to human nontumour brain tissue. The authors suggested that modulation of endocannabinoids in these tumour tissues could be an endogenous antiproliferative mechanism acting through selective cannabinoid receptor activation. Even if this hypothesis is not demonstrated yet, similar mechanisms have been suggested in colon cancer cells by Ligresti *et al.* (2003). The different endocannabinoid metabolism in normal compared to tumour cells and the different effects exerted by endocannabinoids is an unquestionable issue, probably connected with physiological fundamental properties, which could be a possible means to control tumour growth. Finally, it is interesting to remark that cannabinoids cannot induce significant changes in the survival of non-transformed epidermal cell lines MCA3D, HaCat and of primary human keratinocytes, whereas they block *in vivo* the growth of highly malignant PDV.C57-derived tumours (Casanova *et al.*, 2003).

Cannabinoid hydrolysis and reuptake inhibitors

A series of compounds, such as palmitoylethanolamine, might act as 'entourage' substances enhancing cannabinoid biological actions. Di Marzo *et al.* (2001) reported that chronic treatment with PEA enhanced the AEA-induced inhibition of HBCC proliferation decreasing the expression of FAAH, the enzyme mainly responsible for AEA degradation. Similar results were obtained with HU210, which cannot be hydrolysed by FAAH, suggesting that PEA could also enhance the vanilloid VR1 receptor-mediated effects of AEA on calcium influx into cells (De Petrocellis *et al.*, 2000, 2002; Di Marzo *et al.*, 2002). Recent studies in colorectal cancer cells *in vitro* (Ligresti *et al.*, 2003), and in thyroid carcinoma cells *in vitro* and *in vivo* (Bifulco *et al.*, 2004), argue for a therapeutic anticancer strategy aimed at raising the levels of endocannabinoids by preventing their cellular reuptake and enzymatic degradation. VDM11, a selective inhibitor of endocannabinoid cellular reuptake, and arachidonoyl-serotonin (AA-5-HT), a blocker of endocannabinoid enzymatic hydrolysis, both inhibited the *in vitro* growth of rat thyroid-transformed cells (KiMol), and *in vivo* of tumour xenografts induced by subcutaneous injection in mice of the same cell line (Bifulco *et al.*, 2004). Other evidence demonstrated that a decreased 2-AG hydrolysis inhibited invasion of androgen-independent cancer cells (Nithipatikom *et al.*, 2005) and Ben-Shabat *et al.* (1998) showed that 2-acyl-glycerol esters, such as 2-linoleoyl-glycerol (2-LG), potentiated the central biological activity of 2-AG in various normal murine tissues. Given that cannabinoid receptors expression and/or endocannabinoids levels are altered in certain malignancies, as in gliomas, astrocytomas and transformed thyroid epithelium, it would be plausible to argue that endocannabinoids exert a *tonic* control of tumour growth. Thus the inhibitors of cannabinoid inactivation and reuptake might be considered as new tools for therapeutic intervention.

Effects of cannabinoids on tumour progression

Modulation of angiogenesis

Angiogenesis, providing nutrients to proliferating cancer cells, is a critical event involved in the progression of solid tumours.

Positive and negative regulators of angiogenesis could be produced by cancer cells, by vascular endothelial cells, by infiltrating inflammatory cells and by the extracellular matrix (Kuroi & Toi, 2001; Distler *et al.*, 2003).

Increasing evidence suggests that antitumour effect of cannabinoid-related drugs could be at least in part ascribed to inhibition of tumour neoangiogenesis in animal models. The nonpsychoactive CB2-agonist cannabinoid JWH-133 inhibited *in vitro* human umbilical vein endothelial cells (HUVEC) migration and survival (Blazquez *et al.*, 2003); *in vivo* JWH-133 treatment of C6 glioma- and grade IV astrocytoma-derived tumours reduced expression levels of angiopoietin-2 (Ang-2), VEGF, and matrix metalloproteinase-2 (MMP) (Blazquez *et al.*, 2003), three proangiogenic factors that destabilize vessel integrity, facilitate vessel sprouting and endothelial cells growth, disrupte the extracellular matrix organization, respectively. These findings were confirmed by cDNA array analysis showing that JWH-133 administration to mouse downregulated in gliomas genes related to angiogenesis, hypoxia and metastasis and increased the expression of metalloproteinase substrates involved into matrix remodelling, probably *via* ceramide *de novo* synthesis (Blazquez *et al.*, 2004).

Several authors (Rak *et al.*, 1995; Casanova *et al.*, 2002) suggested that oncogenes, such as mutant *ras*, may have an impact on tumour growth and progression through upregulation of VEGF, a common element of the *ras*-dependent angiogenic phenotype (Grunstein *et al.*, 1999). Casanova *et al.* (2003) evaluated the potential antiangiogenetic power of cannabinoids in mouse skin carcinoma cell line (PDV-C57) expressing high levels of activated *ras* and EGF-R and showed that WIN-55,212-2 or JWH-133 were able to arrest *in vivo* the growth of highly malignant PDV-C57 cells-derived tumours: in this model, cannabinoid treatment decreased the expression of proangiogenetic factors VEGF, Ang2 and placental growth factor (PIGF). Similarly, Met-F-AEA, by inhibiting p21^{ras} activity, prevented the growth of v-K-ras-transformed rat thyroid cells both *in vitro* and *in vivo* (Bifulco *et al.*, 2001). Furthermore, it inhibited growth of already established tumours by reducing the expression of both VEGF and its receptor Flt1, and upregulating the levels of the cyclin-dependent kinase inhibitor p27^{kip} (Portella *et al.*, 2003).

Modulation of cancer cell migration and metastasis

Cell migration plays important role in many physiological and pathological processes, including angiogenesis, tissue repair, metastasis and inflammation (Lauffenburger & Horwitz, 1996). The ability to mediate cell migration may be shared by many Gi protein-coupled receptors (Neptune & Bourne, 1997).

Cannabinoid variable effects on cell migration seem to be dependent on both cellular differentiation levels and specific activation of different receptors. Song & Zhong (2000) demonstrated that cannabinoid agonists (HU210, WIN 55212-2, AEA) induced migration of human embrionic kidney 293 cells. The anandamide-induced cell migration was CB1-mediated in human embrionic kidney 293 cells and it was blocked by PD98059 (MAPK inhibitor), suggesting that ERK, rather than adenylate cyclase, was crucial for CB1-mediated migration. On the other hand, the antitumour effects of

cannabidiol (CBD), a nonpsychoactive cannabinoid, could be ascribed, beside to the antiproliferative action on U87 and U373 human glioma cells *in vitro* and *in vivo* (Massi *et al.*, 2004), to inhibition of migration. In U87 cells, such inhibition did not involve classical Gi/o protein-coupled cannabinoid receptors (Vaccani *et al.*, 2005). Moreover, Met-F-AEA was able to inhibit proliferation of a metastasis-derived thyroid cancer cell line, MPTK-6, more efficaciously than of the primary thyroid cancer-derived TK-6 cells (Portella *et al.*, 2003). To test the *in vivo* effects of Met-F-AEA on induction of metastatic foci, the authors used the Lewis lung carcinoma model of metastatic spreading and demonstrated that Met-F-AEA efficaciously interfered with the formation of lung metastatic nodules by acting on CB1 receptors. Recently, our group demonstrated that Met-F-AEA treatment inhibited both adhesion and migration of the highly invasive metastatic breast cancer cell lines MDA-MB-231 and TSA-E1, by *in vitro* testing in an adhesion and migration assay on type IV collagen, the major component of the basement membrane. Furthermore, Met-F-AEA treatment significantly reduced number and dimension of metastatic nodes induced by TSA-E1 cell injection in syngenic mice (Grimaldi *et al.*, 2006).

In androgen-independent prostate cancer cell lines PC3 and DU145, 2-AG reduced invasion through the CB1-dependent inhibition of adenylyl cyclase, decreasing phospho-kinase A (PKA) activity (Nithipatikom *et al.*, 2004). Compounds containing a trifluoromethyl-ketone moiety (TKF), by blocking 2-AG hydrolysis, were able to efficaciously decrease prostate cancer cells spreading (Nithipatikom *et al.*, 2005).

The cannabinoid-modulated migration could finely regulate immunological antitumour responses. Interestingly, in differentiated HL-60 leukemia cells, 2-AG induced a significative production of chemokine (Kishimoto *et al.*, 2004; Sugiura *et al.*, 2004), caused rapid actin rearrangement and morphological changes, such as extension of pseudopods and increased migration (Kishimoto *et al.*, 2003; Gokoh *et al.*, 2005). Moreover, 2-AG stimulated migration of NK cells (Kishimoto *et al.*, 2005), splenocytes, B lymphoid cells and myeloid leukaemia cells (Jorda *et al.*, 2002). 2-AG-induced migration was CB2 receptor-dependent and in B cells was enhanced by CD40 costimulation (Rayman *et al.*, 2004).

Most studies *in vitro* and *in vivo* indicated that THC is immunosuppressive on macrophages, NK cells and T lymphocytes (Bhargava *et al.*, 1996; Klein *et al.*, 1998; McCoy *et al.*, 1999): in murine lung cancer models, THC could promote, rather than suppress, tumour growth inhibiting antitumour immunity by a CB2 receptor-mediated cytokine-dependent patway (enhanced IL-10 and TGFβ, reduced IL2 and IFN-γ) (Zhu *et al.*, 2000).

Noteworthy, AEA alone had no effect on the migration of leukocytes, HL-60 and monocytes (Kishimoto *et al.*, 2003), whereas stimulated embryonic kidney, microglial and myeloid leukaemia cells transfected with the CB-2 receptor gene (Jorda *et al.*, 2003). Moreover, AEA could inhibit chemokine-induced migration of CD8+ T lymphocytes and of SW480 colon carcinoma cells through activation of distinct cannabinoid receptors: CB2 in lymphocytes and CB1 in colon carcinoma cells, respectively, suggesting that specific inhibition of tumour cells migration could be obtained without significant effect on the immune system at least in colon cancer (Joseph *et al.*, 2004).

Multifaceted role of COX-2 and cannabinoids in tumour progression

The enzyme COX catalyses the conversion of arachidonic acid to PGH2, an endoperoxide that functions as precursor of prostaglandins (PGs) and tromboxane (TX). The constitutive isoform, COX-1, is ubiquitous and responsible for physiological functions; COX-2, the isoform expressed by cells involved in inflammation (macrophages, monocytes, platelets) can be dramatically induced by a variety of stimuli (Morita, 2002).

Recent data showed that COX-2-derived prostaglandins modulated the production of proangiogenetic factors in colon cancer cells (Hinz & Brune, 2002), and that COX-2 overexpression could be a common mechanism, identified in a number of epithelial cancer cells (for a review see Prescott & Fitzpatrick, 2000; Romano & Clària, 2003; Zha *et al.*, 2004), resulting in resistance to apoptosis (Tsujii & Dubois, 1995), increased invasiveness (Tsujii *et al.*, 1997) and tumour angiogenesis (Tsujii *et al.*, 1998). Increasing evidence suggested that selective COX-2 inhibitors may represent novel chemopreventive tools (for a review, see Ruegg *et al.*, 2003). A very intriguing hypothesis for the possible role of endocannabinoids on the control of tumour angiogenesis has been proposed by Ligresti *et al.* (2003). They found that 2-AG and AEA concentration was increased in colorectal cancer cells (CRC) compared to normal mucosal tissue and they proposed that these compounds might act as endogenous growth inhibitors through two distinct mechanisms: that is, by stimulation of cannabinoid receptors and by reducing prostaglandins production, since they efficaciously competed with COX-2 substrates (Marnett, 2002). The CB receptor independent effect of anandamide was investigated in colon cancer cell by Patsos *et al.* (2005). They showed that AEA significantly reduced the growth of COX-2-expressing HT29 and HCA7/C29 CRC cell lines and that COX-2 produced metabolites of AEA, prostaglandin-ethanolamines (PG-EAs), which induced apoptosis in CRC cells. Since PG-EAs production was increased in AEA-treated cells and COX-2 selective inhibitors partially attenuated AEA-induced cell death, the authors suggested that a combination of factor, including PG-EAs and COX-2 metabolites, could play a role, at least in part, in the antiproliferative properties of AEA (Patsos *et al.*, 2005). On the other hand, an increased COX-2 expression has been associated with poor prognosis in lung cancer (Achiwa *et al.*, 1999), and it is induced by Methanandamide in murine lung cancer *via* a cannabinoid receptor-independent pathway (Gardner *et al.*, 2003).

Causal relationship between overexpression of COX-2 and carcinogenesis has been demonstrated in breast, colon and in lung cancer. In human breast cancer cell lines, COX-2 and PGE2, the major COX-2 products, were poorly expressed in the MCF-7 cell line and overexpressed in the metastatic cell line MDA-MB-231 (Liu & Rose, 1996); in human breast tumours, a significant correlation between COX-2 expression and aromatase (the enzyme catalysing oestrogen production from androgens) expression has been found (Brueggemeier *et al.*, 1999); finally, clinical studies showed a strong association of high metastatic potential and lack of oestrogen and progesterone receptors with high PGE2 concentration (Rolland *et al.*, 1980).

COX-2 overexpression was detected in human colorectal carcinoma compared with normal epithelium (Eberhart *et al.*, 1994; Elder *et al.*, 2002) and PGE2 was increased in human

colorectal carncer tissue (Rigas *et al*., 1993). Evidence of the role played by COX-2 and PGE2 in precancerous lesions and in cancer growth was provided from clinical (Kune *et al*., 1988; Steinbach *et al*., 2000) and animal (Kawamori *et al*., 1998; Oshima *et al*., 2001) studies.

The nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the selective COX-2 inhibitors (coxibs) have been proposed as anticancer agents. In fact selective COX-2 inhibitors suppressed the growth of human colon and epithelial cancers (for a review, see Koki *et al*., 2002) and they could enhance the response to conventional anticancer therapies (Moore *et al*., 2000; Trifan *et al*., 2002).

Taken together, these data suggested that specific COX-2 inhibitors might be used as adjuvants in the treatment of tumours as well as in cancer prevention. However, COX-2-derived products have a variety of protective properties: the prostacyclin PGI2 exert antioxidant effect which may retard atherogenesis (Pratico *et al*., 1998) and contributes to the atheroprotective effect of oestrogen (Egan *et al*., 2004); PDG2 and PGE2 showed hepato-protective function in a murine model of pharmacological-induced acute liver injury (Reilly *et al*., 2001); adiponectin induced COX-2-dependent synthesis of PGE2 protects the heart from ischemia–reperfusion injury (Shibata *et al*., 2005). In this scenario, some advantage could be offered by the use of endocannabinoids compared to selective COX-2 inhibitors: (a) AEA induced nonapoptotic cell death in high COX-2-expressing colorectal tumour cells (Patsos *et al*., 2005) and in prostate carcinoma cells (Mimeault *et al*., 2003); these properties could be beneficial in treating tumour cells that have become resistant to induction of apoptosis; (b) normal cells which do not express COX-2 were resistent to endocannabinoid induced cell death (Patsos *et al*., 2005); (c) AEA neither increased COX-2 levels nor inhibited its activity, at least in CRC cells, but acting as substrate (Marnett, 2002; Ligresti *et al*., 2003; Patsos *et al*., 2005) it might preserve the protective effects of COX-2-derived products.

Conclusions

Presented findings suggest that cannabinoids exert a number of effects depending on cell types, activation of signal transduction pathways, route of drug administration, timing of drug delivery and, last but not least, responsivity of tumour and normal cells.

Epidemiological studies reported inconsistent association between cannabis smoke and cancer, and administration of high oral doses of THC in rats or mice did not increase tumour incidences in a 2-year study (Chan *et al*., 1996). In animal models, cannabinoids exert a direct antiproliferative effect on tumours, but they could indirectly enhance tumour growth *via* inhibition of immunogenicity (for immunosuppressive effect of cannabinoids, see Klein, 2005). The typical immunosuppressive effect of THC is an unquestionable topic imposing caution in the dosage and administration timing of CB2-receptor-selective compounds (Klein *et al*., 2000; Salzet *et al*., 2000).

The immunosuppressive properties of plant-derived cannabinoids could enhance tumour cell proliferation (Zhu *et al*., 2000; McKallip *et al*., 2005) and accelerate cancer progression in patients, but the biological response to cannabinoids critically depends on drug concentration and cellular context (Hart *et al*., 2004). Nevertheless, different therapeutic strate-

gies could be developed on the basis of peculiar characteristics expressed by several malignancies. Jones & Howl (2003) suggested as therapeutic target for tumour intervention some distinctive properties: (1) in cancer, such as malignant astrocytomas, gliomas, breast, thyroid, prostate, where cannabinoid receptor expression is enhanced, strategies aimed at raising levels of endocannabinoids could be a successfully treatment; (2) in colorectal carcinoma, the increased expression of endocannabinoids suggests that inhibitors of endocannabinoid metabolism could be used as therapeutic tools; (3) upregulation of CB2 receptor expression in malignant astrocytomas and gliomas and/or the increased CB2/CB1 ratio in tumours of immune origin could suggest the use of cannabinoid-based drugs devoid of psycotropic effects. Moreover, there is at present no obvious universal mechanism whereby cannabinoids affect cell viability and proliferation; furthermore, the immunosuppressive properties of cannabinoids or their effects on COX-2 expression, even if incompletely demonstrated to date, could represent *cons* evidence for medical use of cannabinoids, at least in lung carcinoma.

Indeed, cannabinoids have the advantage of being well tolerated in animal studies and they do not present the generalized toxic effects of most conventional chemotherapeutic agents (Guzman *et al*., 2003). Routes of cannabinoids administration have been recently studied. THC is rapidly absorbed after inhalation and its effects become apparent within minutes. Grotenhermen (2001) showed that THC oral administration was associated with slow onset of action and with accidental overdosage. In fact, maximum THC serum concentration measured after smoke intake (Huestis *et al*., 1992) is 2–3-fold higher than maximum serum concentration achievable with oral or rectal THC administration (Consroe *et al*., 1991; Brenneisen *et al*., 1996). The inhalation may have pharmacokinetic advantages, but it requires use of higher potency cannabinoids and strategy aimed at eliminating carcinogenic products combustion: for this purpose, Gieringer (2001) proposed the vaporization lacking the carcinogenic compounds formed during combustion. The trans-dermal route could be eligible for pain, nausea and vomiting treatment in chemotherapy patients giving a continuous steady dose (Stinchcomb *et al*., 2001).

In rats, THC and WIN-55,212-2 administered by infusion at the site of tumour showed a good efficacy, but so far, only preliminary results from one clinical study applying a strategy of local THC administration in patients with recurrent glioblastoma multiforme has been reported (Blazquez *et al*., 2004). Moreover, long-term effects of chronically administered cannabinoids have not been studied. To date, the prescription of cannabinoids is provided for medical conditions that are not adequately controlled by standard treatments, but considering their potentiality in clinical practise the Clinical Cannabinoid Group, chaired by Dr Peterwee, encourage properly conducted clinical trials to evaluate the further potential therapeutic uses of cannabinoids alone or in combination with other drugs.

Even if the use of cannabinoids in clinical practice needs further preclinical research, in order to confirm safety, efficacy, doses and administration protocols, the cannabinoids could provide unquestionable advantages compared to current antitumoural therapies: (1) cannabinoids selectively affect tumour cells more than their nontransformed counterparts that might even be protected from cell death; (2) systematically administered selective inhibitors of endocannabinoid degrada-

tion would be effective only in those tissues where endocannabinoid levels are pathologically altered, without any significant psychotropic or immunosuppressive activity; (3) selective CB1 agonists unable to cross the blood–brain barrier would be deprived of the immunosuppressive and psychotropic effects of cannabinoids and therefore could be efficaciously used as antineoplastic drugs in a large number of tumours, with the exception of glioma; (4) cannabinoids could represent an efficacious therapy in COX-2-expressing tumours that have become resistant to induction of apoptosis: acting as COX-2-substrates with no effect on the protective properties of COX-2-derived products, they could offer some advantage with respect to the NSAID in order to enhance the sensibility to conventional anticancer therapies.

Even if further *in vivo* research are required to clarify cannabinoids action in cancer and especially to test their effectiveness in patients, the cannabinoid system represent a promising target for cancer treatment.

References

ACHIWA, H.., YATABE, Y.., HIDA, T.., KUROISHI, T.., KOZAKI, K.., NAKAMURA, S.., OGAWA, M.., SUGIURA, T.., MITSUDOMI, T.. & TAKAHASHI, T.. (1999). Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. *Clin. Cancer Res.*, **5**, 1001–1005.

BAKER, D.., PRYCE, G.., GIOVANNONI, G.. & THOMPSON, A.J.. (2003). The therapeutic potential of cannabis. *Lancet Neurol.*, **2**, 291–298 Review.

BARSKY, S.H.., ROTH, M.D.., KLEERUP, E.C., SIMMONS, M. & TASHKIN, D.P. (1998). Histopathologic and molecular alterations in bronchial epithelium in habitual smokers of marijuana, cocaine, and/or tobacco. *J. Natl. Cancer Inst.*, **90**, 1198–1205.

BEN-SHABAT, S., FRIDE, E., SHESKIN, T., TAMIRI, T., RHEE, M.H., VOGEL, Z., BISOGNO, T., DE PETROCELLIS, L., DI MARZO, V. & MECHOULAM, R. (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur. J. Pharmacol.*, **353**, 23–31.

BERRENDERO, F., SEPE, N., RAMOS, J.A., DI MARZO, V. & FERNANDEZ-RUIZ, J.J. (1999). Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. *Synapse*, **33**, 181–191.

BHARGAVA, H.N., HOUSE, R.V., THORAT, S.N. & THOMAS, P.T. (1996). Cellular immune fuction in mice tolerant to or abstinent from 1-*trans*-*Δ*-9-tetrahydrocannabinol. *Pharmacology*, **52**, 271.

BIFULCO, M. & DI MARZO, V. (2002). Targeting the endocannabinoid system in cancer therapy: a call for further research. *Nat. Med.*, **8**, 547–550.

BIFULCO, M., LAEZZA, C., PORTELLA, G., VITALE, M., ORLANDO, P., DE PETROCELLIS, L. & DI MARZO, V. (2001). Control by the endogenous cannabinoid system of ras oncogene-dependent tumor growth. *FASEB J.*, **15**, 2745–2747.

BIFULCO, M., LAEZZA, C., VALENTI, M., LIGRESTI, A., PORTELLA, G. & DI MARZO, V. (2004). A new strategy to block tumor growth by inhibiting endocannabinoid inactivation. *FASEB J.*, **18**, 1606–1608.

BLAZQUEZ, C., CASANOVA, M.L., PLANAS, A., DEL PULGAR, T.G., VILLANUEVA, C., FERNANDEZ-ACENERO, M.J., ARAGONES, J., HUFFMAN, J.W., JORCANO, J.L. & GUZMAN, M. (2003). Inhibition of tumor angiogenesis by cannabinoids. *FASEB J.*, **17**, 529–531.

BLAZQUEZ, C., GONZALEZ-FERIA, L., ALVAREZ, L., HARO, A., CASANOVA, M.L. & GUZMAN, M. (2004). Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. *Cancer Res.*, **64**, 5617–5623.

BOUABOULA, M., POINOT-CHAZEL, C., BOURRIE, B., CANAT, X., CALANDRA, B., RINALDI-CARMONA, M., LE FUR, G. & CASELLAS, P. (1995). Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem. J.*, **312** (Part 2), 637–641.

BRENNEISEN, R., EGLI, A., ELSOHLY, M.A., HENN, V. & SPIESS, Y. (1996). The effect of orally and rectally administered Δ9-tetrahydrocannabinol on spasticity: a pilot study with 2 patients. *Int. J. Clin. Pharmacol. Ther.*, **34**, 446–452.

BRUEGGEMEIER, R.W., QUINN, A.L., PARRETT, M.L., JOARDER, F.S., HARRIS, R.E. & ROBERTSON, F.M. (1999). Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens. *Cancer Lett.*, **140**, 27–35.

CARAYON, P., MARCHAND, J., DUSSOSSOY, D., DEROCQ, J.M., JBILO, O., BORD, A., BOUABOULA, M., GALIEGUE, S., MONDIERE, P., PENARIER, G., FUR, G.L., DEFRANCE, T. & CASELLAS, P. (1998). Modulation and functional involvement of CB2 peripheral cannabinoid receptors during B-cell differentiation. *Blood*, **92**, 3605–3615.

CARRACEDO, A., GEELEN, M.J., DIEZ, M., HANADA, K., GUZMAN, M. & VELASCO, G. (2004). Ceramide sensitizes astrocytes to oxidative stress: protective role of cannabinoids. *Biochem J.*, **380** (Part 2), 435–440.

CASANOVA, M.L., BLAZQUEZ, C., FERNANDEZ-ACENERO, M.J., VILLANUEVA, C., JORCANO, J. & GUZMAN, M. (2001). *CB1 and CB2 receptors are expressed in the skin and their activation inhibits the growth of skin cancer cells,* Symposium on Cannabinoids, Vol. 1, p. 151, International Cannabinoid Research Society: Burlington, VT.

CASANOVA, M.L., BLAZQUEZ, C., MARTINEZ-PALACIO, J., VILLANUEVA, C., FERNANDEZ-ACENERO, M.J., HUFFMAN, J.W., JORCANO, J.L. & GUZMAN, M. (2003). Inhibition of skin tumor growth and angiogenesis *in vivo* by activation of cannabinoid receptors. *J. Clin. Invest.*, **111**, 43–50.

CASANOVA, M.L., LARCHER, F., CASANOVA, B., MURILLAS, R., FERNANDEZ-ACENERO, M.J., VILLANUEVA, C., MARTINEZ-PALACIO, J., ULLRICH, A., CONTI, C.J. & JORCANO, J.L. (2002). A critical role for ras-mediated, epidermal growth factor receptor-dependent angiogenesis in mouse skin carcinogenesis. *Cancer Res.*, **62**, 3402–3407.

CHAN, P.C., SILLS, R.C., BRAUN, A.G., HASEMAN, J.K. & BUCHER, J.R. (1996). Toxicity and carcinogenicity of delta 9-tetrahydrocannabinol in Fischer rats and B6C3F1 mice. *Fundam. Appl. Toxicol.*, **30**, 109–117.

CLEVENGER, C.V., CHANG, W.P., NGO, W., PASHA, T.L., MONTONE, K.T. & TOMASZEWSKI, J.E. (1995). Expression of prolactin and prolactin receptor in human breast carcinoma. Evidence for an autocrine/paracrine loop. *Am. J. Pathol.*, **146**, 695–705.

CONSROE, P., KENNEDY, K. & SCHRAM, K. (1991). Assay of plasma cannabidiol by capillary gas chromatography ion trap mass spectroscopy following high-dose repeated daily oral administration in humans. *Pharmacol. Biochem. Behav.*, **40**, 517–522.

CORASANITI, M.T., STRONGOLI, M.C., PICCIRILLI, S., NISTICO, R., COSTA, A., BILOTTA, A., TURANO, P., FINAZZI-AGRO, A. & BAGETTA, G. (2000). Apoptosis induced by gp120 in the neocortex of rat involves enhanced expression of cyclooxygenase type 2 and is prevented by NMDA receptor antagonists and by the 21-aminosteroid U-74389G. *Biochem. Biophys. Res. Commun.*, **274**, 664–669.

COTA, D., MARSICANO, G., TSCHOP, M., GRUBLER, Y., FLACHSKAMM, C., SCHUBERT, M., AUER, D., YASSOURIDIS, A., THONE-REINEKE, C., ORTMANN, S., TOMASSONI, F., CERVINO, C., NISOLI, E., LINTHORST, A.C., PASQUALI, R., LUTZ, B., STALLA, G.K. & PAGOTTO, U. (2003). The endogenous cannabinoid system affects energy balance *via* central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.*, **112**, 423–431.

DE PETROCELLIS, L., BISOGNO, T., DAVIS, J.B., PERTWEE, R.G. & DI MARZO, V. (2000). Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. *FEBS Lett.*, **483**, 52–56.

DE PETROCELLIS, L., BISOGNO, T., LIGRESTI, A., BIFULCO, M., MELCK, D. & DI MARZO, V. (2002). Effect on cancer cell proliferation of palmitoylethanolamide, a fatty acid amide interacting with both the cannabinoid and vanilloid signalling systems. *Fundam. Clin. Pharmacol.,* **16,** 297–302.

DE PETROCELLIS, L., MELCK, D., PALMISANO, A., BISOGNO, T., LAEZZA, C., BIFULCO, M. & DI MARZO, V. (1998). The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 8357–8380.

DEVANE, W.A., HANUS, L., BREUER, A., PERTWEE, R.G., STEVENSON, L.A., GRIFFIN, G., GIBSON, D., MANDELBAUM, A., ETINGER, A. & MECHOULAM, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science,* **258,** 1946–1949.

DI MARZO, V. (1998). Endocannabinoids and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. *Biochim. Biophys. Acta,* **1392,** 153–175.

DI MARZO, V., BIFULCO, M. & DE PETROCELLIS, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov.,* **3,** 771–784 Review.

DI MARZO, V., BREIVOGEL, C.S., TAO, Q., BRIDGEN, D.T., RAZDAN, R.K., ZIMMER, A.M., ZIMMER, A. & MARTIN, B.R. (2000). Levels, metabolism, and pharmacological activity of anandamide in CB(1) cannabinoid receptor knockout mice: evidence for non-CB(1), non-CB(2) receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.,* **75,** 2434–2444.

DI MARZO, V., DE PETROCELLIS, L., FEZZA, F., LIGRESTI, A. & BISOGNO, T. (2002). Anandamide receptors. *Prostaglandins Leukot Essent Fatty Acids,* **66,** 377–391.

DI MARZO, V., MELCK, D., ORLANDO, P., BISOGNO, T., ZAGOORY, O., BIFULCO, M., VOGEL, Z. & DE PETROCELLIS, L. (2001). Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. *Biochem. J.,* **358,** 249–255.

DISTLER, J.H., HIRTH, A., KUROWSKA-STOLARSKA, M., GAY, R.E., GAY, S. & DISTLER, O. (2003). Angiogenic and angiostatic factors in the molecular control of angiogenesis. *Q. J. Nucl. Med.,* **47,** 149–161 Review.

EBERHART, C.E., COFFEY, R.J., RADHIKA, A., GIARDIELLO, F.M., FERRENBACH, S. & DUBOIS, R.N. (1994). Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology,* **107,** 1183–1188.

EGAN, K.M., LAWSON, J.A., FRIES, S., KOLLER, B., RADER, D.J., SMYTH, E.M. & FITZGERALD, G.A. (2004). COX-2 derived prostacyclin confers atheroprotection on female mice. *Science,* **306,** 1954–1957.

ELDER, D.J., BAKER, J.A., BANU, N.A., MOORGHEN, M. & PARASKEVA, C. (2002). Human colorectal adenomas demonstrate a size-dependent increase in epithelial cyclooxygenase-2 expression. *J. Pathol.,* **198,** 428–434.

ELLERT-MIKLASZEWSKA, A., KAMINSKA, B. & KONARSKA, L. (2005). Cannabinoids down-regulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. *Cell Signal.,* **17,** 25–37.

FELDER, C.C. & GLASS, M. (1998). Cannabinoid receptors and their endogenous agonists. *Annu. Rev. Pharmacol. Toxicol.,* **38,** 179–200 Review.

GALIEGUE, S., MARY, S., MARCHAND, J., DUSSOSSOY, D., CARRIERE, D., CARAYON, P., BOUABOULA, M., SHIRE, D., LE FUR, G. & CASELLAS, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.,* **232,** 54–61.

GALVE-ROPERH, I., SANCHEZ, C., CORTES, M.L., DEL PULGAR, T.G., IZQUIERDO, M. & GUZMAN, M. (2000). Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nat. Med.,* **6,** 313–319.

GARDNER, B., ZHU, L.X., SHARMA, S., TASHKIN, D.P. & DUBINETT, S.M. (2003). Methanandamide increases COX-2 expression and tumor growth in murine lung cancer. *FASEB J.,* **17,** 2157–2159.

GIERINGER, D. (2001). Cannabis 'vaporization': a promising strategy for smoke harm reduction. *J. Cannabis Ther.,* **1,** 153–170.

GOKOH, M., KISHIMOTO, S., OKA, S., MORI, M., WAKU, K., ISHIMA, Y. & SUGIURA, T. (2005). 2-Arachidonoylglycerol, an endogenous cannabinoid receptor ligand, induces rapid actin polymerization in HL-60 cells differentiated into macrophage-like cells. *Biochem J.,* **386,** 583–589.

GOMEZ DEL PULGAR, T., VELASCO, G., SANCHEZ, C., HARO, A. & GUZMAN, M. (2002). De novo-synthesized ceramide is involved in cannabinoid-induced apoptosis. *Biochem J.,* **363,** 183–188.

GRIMALDI, C., PISANTI, S., LAEZZA, C., MALFITANO, A.M., SANTORO, A., VITALE, M., CARUSO, M.G., NOTARNICOLA, M., IACUZZO, I., PORTELLA, G., DI MARZO, V. & BIFULCO, M. (2006). Anandamide inhibits adhesion and migration of breast cancer cells. *Exp. Cell. Res.,* **312,** 363–373.

GROTENHERMEN, F. (2001). Harm reduction associated with inhalation and oral administration of cannabis and THC. *J. Cannabis Ther.,* **1,** 133–152.

GRUNSTEIN, J., ROBERTS, W.G., MATHIEU-COSTELLO, O., HANAHAN, D. & JOHNSON, R.S. (1999). Tumor-derived expression of vascular endothelial growth factor is a critical factor in tumor expansion and vascular function. *Cancer Res.,* **59,** 1592–1598.

GUZMAN, M. (2003). Cannabinoids: potential anticancer agents. *Nat. Rev. Cancer,* **3,** 745–755.

GUZMAN, M., GALVE-ROPERH, I. & SANCHEZ, C. (2001a). Ceramide: a new second messenger of cannabinoid action. *Trends Pharmacol. Sci.,* **22,** 19–22 Review.

GUZMAN, M., SANCHEZ, C. & GALVE-ROPERH, I. (2001b). Control of the cell survival/death decision by cannabinoids. *J. Mol. Med.,* **78,** 613–625.

GUZMAN, M., SANCHEZ, C. & GALVE-ROPERH, I. (2002). Cannabinoids and cell fate. *Pharmacol. Ther.,* **95,** 175–184 Review.

HALL, W., MACDONALD, C. & CURROW, D. (2005). Cannabinoids and cancer: causation, remediation, and palliation. *Lancet Oncol.,* **6,** 35–42 Review.

HALL, W.D. & MACPHEE, D. (2002). Cannabis use and cancer. *Addiction,* **97,** 243–247.

HAMELERS, I.H.L., VANSCHAIK, R.F.M.A., SUSSENBACK, J.S. & STEENBERGH, P.H. (2003). 17b-Estradiol responsiveness of MCF-7 laboratory strains is dependent on an autocrine signal activating the IGF type I receptor. *Cancer Cell Int.,* **3,** 10–20.

HART, S., FISCHER, O.M. & ULLRICH, A. (2004). Cannabinoids induce cancer cell proliferation *via* tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Res.,* **64,** 1943–1950.

HINZ, B. & BRUNE, K. (2002). Cyclooxygenase-2–10 years later. *J. Pharmacol. Exp. Ther.,* **300,** 367–375 Review.

HINZ, B., RAMER, R., EICHELE, K., WEINZIERL, U. & BRUNE, K. (2004). R(+)-methanandamide-induced cyclooxygenase-2 expression in H4 human neuroglioma cells: possible involvement of membrane lipid rafts. *Biochem. Biophys. Res. Commun.,* **324,** 621–626.

HOWLETT, A.C., BARTH, F., BONNER, T.I., CABRAL, G., CASELLAS, P., DEWANE, W.A., FELDE, C.C., HERKENHAM, M., MACKIE, K., MARTIN, B.R., MECHOULAM, R. & PERTWEE, R.G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.,* **54,** 161–202.

HOWLETT, A.C., QUALY, J.M. & KHACHATRIAN, L.L. (1986). Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol. Pharmacol.,* **29,** 307–313.

HUESTIS, M.A., SAMPSON, A.H., HOLICKY, B.J., HENNINGFIELD, J.E. & CONC, E.J. (1992). Characterization of the absorption phase of marijuana smoking. *Clin. Pharmacol. Ther.,* **52,** 31–41.

JACOBSSON, S.O., WALLIN, T. & FOWLER, C.J. (2001). Inhibition of rat C6 glioma cell proliferation by endogenous and synthetic cannabinoids. Relative involvement of cannabinoid and vanilloid receptors. *J. Pharmacol. Exp. Ther.,* **299,** 951–959.

JONES, S. & HOWL, J. (2003). Cannabinoid receptor systems: therapeutic targets for tumour intervention. *Exp. Opin. Ther. Targets,* **7,** 749–758.

JORDA, M.A., RAYMAN, N., TAS, M., VERBAKEL, S.E., BATTISTA, N., VAN LOM, K., LOWENBERG, B., MACCARRONE, M. & DELWEL, R. (2004). The peripheral cannabinoid receptor Cb2, frequently expressed on AML blasts, either induces a neutrophilic differentiation block or confers abnormal migration properties in a ligand-dependent manner. *Blood,* **104,** 526–534.

JORDA, M.A., RAYMAN, N., VALK, P., DE WEE, E. & DELWEL, R. (2003). Identification characterization and function of a novel oncogene: the peripheral cannabinoid receptor Cb2. *Ann. NY Acad. Sci.*, **996**, 10–16.

JORDA, M.A., VERBAKEL, S.E., VALK, P.J.M., VANKAN-BERKHOUDT, Y.V., MACCARRONE, M., FINAZZI-AGRO', A., LOWENBERG, B. & DELWEL, R. (2002). Hematopoietic cells expressing the peripheral cannabinoid receptor migrate in response to endocannabinoid 2-arachidonoylglycerol. *Blood*, **99**, 2786–2793.

JOSEPH, J., NIGGEMANN, B., ZAENKER, K.S. & ENTSCHLADEN, F. (2004). Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. *Cancer Immunol. Immunother.*, **53**, 723–728.

JOY, J.E., WATSON JR, S.J. & BENSON JR, J.A. eds. (1999). *Marijuana and Medicine-assessing the Science Base.* Washington, D.C.: Institute of Medicine-National Academy Press.

KAWAMORI, T., RAO, C.V., SEIBERT, K. & REDDY, B.S. (1998). Chemopreventive activity of celecoxib, a specific cyclo-oxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res.*, **58**, 409–412.

KEARN, C.S. & HILLARD, C.J. (1997). *Rat microglial cells express the peripheral-type cannabinoid receptor (CB2) which is negatively coupled to adenylyl cyclase,* Symposium on Cannabinoids, Vol. 1, p. 61, International Cannabinoid Research Society: Burlington, VT.

KIM, H.G., KASSIS, J., SOUTO, J.C., TURNER, T. & WELLS, A. (1999). EGF receptor signaling in prostate morphogenesis and tumorigenesis. *Histol. Histopathol.*, **14**, 1175–1182.

KISHIMOTO, S., GOKOH, M., OKA, S., MURAMATSU, M., KAJIWARA, T., WAKU, K. & SUGIURA, T. (2003). 2-Arachidonoylglycerol induces the migration of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes through the cannabinoid CB2 receptor-dependent mechanism. *J. Biol. Chem.*, **278**, 24469–24475.

KISHIMOTO, S., KOBAYASHI , Y., OKA, S., GOKOH, M., WAKU, K. & SUGIURA, T. (2004). 2-Arachidonoylglycerol, an endogenous cannabinoid receptor ligand, induces accelerated production of chemokines in HL-60 cells. *J. Biochem. (Tokyo)*, **135**, 517–524.

KISHIMOTO, S., MURAMATSU, M., GOKOH, M., OKA, S., WAKU, K. & SUGIURA, T. (2005). Endogenous cannabinoid receptor ligand induces the migration of human natural killer cells. *J. Biochem. (Tokyo)*, **137**, 217–223.

KLEIN, T.W. (2005). Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat. Rev. Immunol.*, **5**, 400–411.

KLEIN, T.W., LANE, B., NEWTON, C.A. & FRIEDMAN, H. (2000). The cannabinoid system and cytokine network. *Proc. Soc. Exp. Biol. Med.*, **225**, 1–8 Review.

KLEIN, T.W., NEWTON, C. & FRIEDMAN, H. (1998). Cannabinoid receptors and immunity. *Immunol. today*, **19**, 373.

KOKI, A.T., KHAN, N.K., WOERNER, B.M., SEIBERT, K., HARMON, J.L., DANNENBERG, A.J., SOSLOW, R.A. & MASFERRER, J.L. (2002). Characterization of cyclooxygenase-2 (COX-2) during tumorigenesis in human epithelial cancers: evidence for potential clinical utility of COX-2 inhibitors in epithelial cancer. *Prostaglandins Leukot. Essent. Fatty Acids,* **66**, 13–18.

KUNE, G.A., KUNE, S. & WATSON, L.F. (1988). Colorectal cancer risk, chronic illnesses, operations and medications: a case control results from the Melbourne colorectal cancer study. *Cancer Res.*, **48**, 4399–4404.

KUNOS, G., JARAI, Z., BATKAI, S., GOPARAJU, S.K., ISHAC, E.J., LIU, J., WANG, L. & WAGNER, J.A. (2000). Endocannabinoids as cardiovascular modulators. *Chem. Phys. Lipids*, **108**, 159–168 Review.

KUNOS, G. & PACHER, P. (2004). Cannabinoids cool the intestine. *Nat. Med.*, **10**, 678–679.

KUROI, K. & TOI, M. (2001). Circulating angiogenesis regulators in cancer patients. *Int. J. Biol. Markers*, **16**, 5–26.

LAUFFENBURGER, D.A. & HORWITZ, A.F. (1996). Cell migration: a physically integrated molecular process. *Cell*, **84**, 359–369 Review.

LIGRESTI, A., BISOGNO, T., MATIAS, I., DE PETROCELLIS, L., CASCIO, M.G., COSENZA, V., D'ARGENIO, G., SCAGLIONE, G., BIFULCO, M., SORRENTINI, I. & DI MARZO, V. (2003). Possible endocannabinoid control of colorectal cancer growth. *Gastroenterology*, **125**, 677–687.

LIU, J., GAO, B., MIRSHAHI, F., SANYAL, A.J., KHANOLKAR, A.D., MAKRIYANNIS, A. & KUNOS, G. (2000). Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochem J.*, **346**, 835–840.

LIU, X.H. & ROSE, D.P. (1996). Differential expression and regulation of cyclooxygenase-1 and -2 in two human breast cancer cell lines. *Cancer Res.*, **56**, 5125–5127.

LIU, X.H., WILEY, H.S. & MEIKLEAW (1993). Androgens regulate proliferation of human prostate cancer cells in culture by increasing transforming growth factor-alpha (TGF-alpha) and epidermal growth factor (EGF)/TGF-alpha receptor. *J. Clin. Endocrinol. Metabol.*, **77**, 1472–1478.

LYNN, A.B. & HERKENHAM, M. (1994). Localization of cannabin receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. *J. Pharmacol. Exp. Ther.*, **268**, 1612–1623.

MACCARRONE, M. & FINAZZI-AGRÒ, A. (2002). Endocannabinoids and their actions. *Vitam. Horm.*, **65**, 225–255.

MACCARRONE, M., LORENZON, T., BARI, M., MELINO, G. & FINAZZI-AGRÒ, A. (2000). Anandamide induces apoptosis in human cells *via* vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *J. Biol. Chem.*, **275**, 31938–31945.

MACDONALD, J.C. & VAUGHAN, C.W. (2001). Cannabinoids act backwards. *Nature*, **410**, 527–530.

MACKIE, K. & HILLE, B. (1992). Cannabinoids inhibit *N*-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 3825–3829.

MACPHEE, D. (1999). Effects of marijuana on cell nuclei: a review of the literature relating to the genotoxicity of cannabis. In: *The Health Effects of Cannabis,* ed. Kalant H, Corrigall W, Hall WD & Smart R. pp. 435–458. Toronto: Centre for Addiction and Mental Health.

MARKER, P.C., DONJACOUR, A.A., DAHIYA, R. & CUNHA, G.R. (2003). Hormonal, cellular, and molecular control of prostatic development. *Dev. Biol.*, **253**, 165–174 Review.

MARNETT, L.J. (2002). Recent developments in cyclooxygenase inhibition. *Prostaglandins Other Lipid Mediat.*, **68**, 153–164.

MARSELOS, M. & KARAMANAKOS, P. (1999). Mutagenicity, developmental toxicity and carcinogeneity of cannabis. *Addict Biol.*, **4**, 5–12.

MASSI, P., VACCANI, A., CERUTI, S., COLOMBO, A., ABBRACCHIO, M.P. & PAROLARO, D. (2004). Antitumor effects of cannabidiol, a nonpsychoactive cannabinoid, on human glioma cell lines. *J. Pharmacol. Exp. Ther.*, **308**, 838–845.

MATSUDA, L.A., LOLAIT, S.J., BROWNSTEIN, M.J., YOUNG, A.C. & BONNER, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, **346**, 561–564.

MCALLISTER, S.D., CHAN, C., TAFT, R.J., LUU, T., ABOOD, M.E., MOORE, D.H., ALDAPE, K. & YOUNT, G. (2005). Cannabinoids selectively inhibit proliferation and induce death of cultured human glioblastoma multiforme cells. *J. Neurooncol.*, **74**, 31–40.

MCALLISTER, S.D. & GLASS, M. (2002). CB(1) and CB(2) receptor-mediated signalling: a focus on endocannabinoids. *Prostaglandins Leukot Essent Fatty Acids*, **66**, 161–171.

MCCOY, K.L., MATVEYEVA, M., CARLISLE, S.J. & CABRAL, G.A. (1999). Cannabinoid inhibition of the processing of intact lysozyme by macrophages: evidence for CB2 receptor participation. *J. Pharmacol. Exp. Ther.*, **289**, 1620.

MCKALLIP, R.J., NAGARKATTI, M. & NAGARKATTI, P.S. (2005). Delta-9-tetrahydrocannabinol enhances breast cancer growth and metastasis by suppression of the antitumor immune response. *J. Immunol.*, **174**, 3281–3289.

MECHOULAM, R., BEN-SHABAT, S., HANUS, L., LIGUMSKY, M., KAMINSKI, N.E., SCHATZ, A.R., GOPHER, A., ALMOG, S., MARTIN, B.R., COMPTON, D.R., PERTWEE, R.G., GRIFFIN, G., BAYEWITCH, M., BARG, J. & VOGEL, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.*, **50**, 83–90.

MECHOULAM, R., PANIKASHVILI, D. & SHOHAMI, E. (2002). Cannabinoids and brain injury: therapeutic implications. *Trends Mol. Med.*, **8**, 58–61.

MELCK, D., BISOGNO, T., DE PETROCELLIS, L., CHUANG, H., JULIUS, D., BIFULCO, M. & DI MARZO, V. (1999a). Unsaturated long-chain *N*-acyl-vanillyl-amides (*N*-AVAMs): vanilloid receptor ligands that inhibit anandamide-facilitated transport and bind to CB1 cannabinoid receptors. *Biochem. Biophys. Res. Commun.*, **262**, 275–284.

MELCK, D., DE PETROCELLIS, L., ORLANDO, P., BISOGNO, T., LAEZZA, C., BIFULCO, M. & DI MARZO, V. (2000). Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology*, **141**, 118–126.

MELCK, D., RUEDA, D., GALVE-ROPERH, I., DE PETROCELLIS, L., GUZMAN, M. & DI MARZO, V. (1999b). Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. *FEBS Lett.*, **463**, 235–240.

MENDIZABAL, V.E. & ADLER-GRASCHINSKY, E. (2003). Cannabinoid system as a potential target for drug development in the treatment of cardiovascular disease. *Curr. Vasc. Pharmacol.*, **1**, 301–313 Review.

MIMEAULT, M., POMMERY, N., WATTEZ, N., BAILLY, C. & HENICHART, JP. (2003). Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. *Prostate*, **56**, 1–12.

MOLINA-HOLGADO, E., VELA, J.M., AREVALO-MARTIN, A., ALMAZAN, G., MOLINA-HOLGADO, F., BORRELL, J. & GUAZA, C. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J. Neurosci.*, **22**, 9742–9753.

MONTGOMERY, B.T., YOUNG, C.Y., BILHARTZ, D.T., ANDREWS, P.E., PRESCOTT, J.L., THOMPSON, N.F. & TINDALL, D.J. (1992). Hormonal regulation of prostate-specific antigen (PSA) glycoprotein in the human prostatic adenocarcinoma cell line, LNCaP. *Prostate*, **21**, 63–73.

MOORE, R.J., ZWEIFEL, B.S., HEUVELMAN, D.M., LEABY, K.M., EDWARDS, D.A. & WOENER, B.M. (2000). Enhanced antitumor activity by co-administration of celecoxib and the chemotherapeutic agents cyclophosphamide and 5-FU. *Proc. Am. Assoc. Cancer Res.*, **41**, 409.

MORITA, I. (2002). Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat.*, **68**, 165–175.

MUNRO, S., THOMAS, K.L. & ABU-SHAAR, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, **365**, 61–65.

NA, H.K. & SURH, Y.J. (2002). Induction of cyclooxygenase-2 in Ras-transformed human mammary epithelial cells undergoing apoptosis. *Ann. NY Acad. Sci.*, **973**, 153–160.

NEPTUNE, E.R. & BOURNE, H.R. (1997). Receptors induce chemotaxis by releasing the betagamma subunit of Gi, not by activating Gq or Gs. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 14489–14494.

NITHIPATIKOM, K., ENDSLEY, M.P., ISBELL, M.A., FALCK, J.R., IWAMOTO, Y., HILLARD, C.J. & CAMPBELL, W.B. (2004). 2-Arachidonoylglycerol: a novel inhibitor of androgen-independent prostate cancer cell invasion. *Cancer Res.*, **64**, 8826–8830.

NITHIPATIKOM, K., ENDSLEY, M.P., ISBELL, M.A., WHEELOCK, C.E., HAMMOCK, B.D. & CAMPBELL, W.B. (2005). A new class of inhibitors of 2-arachidonoylglycerol hydrolysis and invasion of prostate cancer cells. *Biochem. Biophys. Res. Commun.*, **332**, 1028–1033.

NUNEZ, E., BENITO, C., PAZOS, M.R., BARBACHANO, A., FAJARDO, O., GONZALEZ, S., TOLON, R.M. & ROMERO, J. (2004). Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. *Synapse*, **53**, 208–213.

OSHIMA, M., MURAI, N., KARGMAN, S., ARGUELLO, M., LUK, P., KWONG, E., TAKETO, M.M. & EVANS, J.F. (2001). Chemoprevention of intestinal polyposis in the APC(delta)716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. *Cancer Res.*, **61**, 1733–1740.

PATSOS, H.A., HICKS, D.J., DOBSON, R.R., GREENHOUGH, A., WOODMAN, N., LANE, J.D., WILLIAMS, A.C. & PARASKEVA, C. (2005). The endogenous cannabinoid, anandamide, induces cell death in colorectal carcinoma cells: a possible role for cyclooxygenase-2. *Gut*, **54**, 1741–1750.

PENG, D., FAN, Z., LU, Y., DEBLASIO, T., SCHER, H. & MENDELSOHN, J. (1996). Antiepidermal growth factor receptor monoclonal antibody 225 upregulates p27KiP1 and induces G1 arrest in prostatic carcinoma cell line DU-145. *Cancer Res.*, **56**, 3666–3669.

PERTWEE, R.G. (2000). Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. *Expert. Opin. Investig. Drugs*, **9**, 1553–1571.

PETERSEN, G., MOESGAARD, B., SCHMID, P.C., SCHMID, H.H., BROHOLM, H., KOSTELJANETZ, M. & HANSEN, H.S. (2005). Endocannabinoid metabolism in human glioblastomas and meningiomas compared to human non-tumour brain tissue. *J. Neurochem.*, **93**, 299–309.

PIOMELLI, D., GIUFFRIDA, A., CALIGNANO, A. & RODRIGUEZ DE FONSECA, F. (2000). The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol. Sci.*, **21**, 218–224.

PORTELLA, G., LAEZZA, C., LACCETTI, P., DE PETROCELLIS, L., DI MARZO, V. & BIFULCO, M. (2003). Inhibitory effects of cannabinoid CB1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. *FASEB J.*, **17**, 1771–1773.

PORTER, A.C. & FELDER, C.C. (2001). The endocannabinoid nervous system: unique opportunities for therapeutic intervention. *Pharmacol. Ther.*, **90**, 45–60.

PRATICO, D., TANGIRALA, R.K., RADER, D.J., ROKACH, J. & FITZGERALD, G.A. (1998). Vitamin E suppresses isoprostane generation *in vivo* and reduces atherosclerosis in ApoE-deficient mice. *Nat. Med.*, **4**, 1189–1192.

PRESCOTT, S.M. & FITZPATRICK, F.A. (2000). Cyclooxygenase-2 and carcinogenesis. *Biochim. Biophys. Acta*, **1470**, 69–78 Review.

RAK, J., MITSUHASHI, Y., BAYKO, L., FILMUS, J., SHIRASAWA, S., SASAZUKI, T. & KERBEL, R.S. (1995). Mutant ras oncogenes upregulate VEGF/VPF expression: implications for induction and inhibition of tumor angiogenesis. *Cancer Res.*, **55**, 4575–4580.

RAMER, R., WEINZIERL, U., SCHWIND, B., BRUNE, K. & HINZ, B. (2003). Ceramide is involved in r(+)-methanandamide-induced cyclooxygenase-2 expression in human neuroglioma cells. *Mol. Pharmacol.*, **64**, 1189–1198.

RAYMAN, N., LAM, K.H., LAMAN, J.D., SIMONS, P.J., LOWENBERG, B., SONNEVELD, P. & DELWEL, R. (2004). Distinct expression profiles of the peripheral cannabinoid receptor in lymphoid tissues depending on receptor activation status. *The J. Immunol.*, **172**, 2111–2117.

REILLY, T.P., BRADY, J.N., MARCHICK, M.R., BOURDI, M., GEORGE, J.W., RADONOVICH, M.F., PISE-MASISON, C.A. & POHL, L.R. (2001). A protective role for cyclooxigenase-2 in drug induced liver injury in mice. *Chem. Res. Toxicol.*, **14**, 1620–1628.

RIGAS, B., GOLDMAN, I.S. & LEVINE, L. (1993). Altered eicosanoid levels in human colon cancer. *J. Lab. Clin. Med.*, **122**, 518–523.

ROLLAND, P.H., MARTIN, P.M., JACQUEMIER, J., ROLLAND, A.M. & TOGA, M. (1980). Prostaglandin in human breast cancer: evidence suggesting that an elevated prostaglandin production is a marker of high metastatic potential for neoplastic cells. *J. Natl. Cancer Inst.*, **64**, 1061–1070.

ROMANO, M. & CLÀRIA, J. (2003). Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy. *FASEB J.*, **17**, 1986–1995.

ROTH, M.D., MARQUES-MAGALLANES, J.A., YUAN, M., SUN, W., TASHKIN, D.P. & HANKINSON, O. (2001). Induction and regulation of the carcinogen-metabolizing enzyme CYP1A1 by marijuana smoke and delta (9)-tetrahydrocannabinol. *Am. J. Resp. Cell Mol. Biol.*, **24**, 339–344.

RUEGG, C., ZARIC, J. & STUPP, R. (2003). Non steroidal anti-inflammatory drugs and COX-2 inhibitors as anti-cancer therapeutics: hypes, hopes and reality. *Ann. Med.*, **35**, 476–487 Review.

RUIZ, L., MIGUEL, A. & DIAZ-LAVIADA, I. (1999). Delta9-tetrahydrocannabinol induces apoptosis in human prostate PC-3 cells *via* a receptor-independent mechanism. *FEBS Lett.*, **458**, 400–404.

SALZET, M., BRETON, C., BISOGNO, T. & DI MARZO, V. (2000). Comparative biology of the endocannabinoid system possible role in the immune response. *Eur. J. Biochem.*, **267**, 4917–4927 Review.

SANCHEZ, C., DE CEBALLOS, M.L., DEL PULGAR, T.G., RUEDA, D., CORBACHO, C., VELASCO, G., GALVE-ROPERH, I., HUFFMAN, J.W., RAMON Y CAJAL, S. & GUZMAN, M. (2001a). Inhibition of glioma growth *in vivo* by selective activation of the CB(2) cannabinoid receptor. *Cancer Res.*, **61**, 5784–5789.

SANCHEZ, C., GALVE-ROPERH, I., CANOVA, C., BRACHET, P. & GUZMAN, M. (1998). Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. *FEBS Lett.*, **436**, 6–10.

SANCHEZ, C., RUEDA, D., SEGUI, B., GALVE-ROPERH, I., LEVADE, T. & GUZMAN, M. (2001b). The CB(1) cannabinoid receptor of astrocytes is coupled to sphingomyelin hydrolysis through the adaptor protein fan. *Mol. Pharmacol.,* **59,** 955–959.

SANCHEZ, M.G., SANCHEZ, A.M., RUIZ-LLORENTE, L. & DIAZ-LAVIADA, I. (2003). Enhancement of androgen receptor expression induced by (*R*)-methanandamide in prostate LNCaP cells. *FEBS Lett.,* **555,** 561–566.

SARFARAZ, S., AFAQ, F., ADHAMI, V.M. & MUKHTAR, H. (2005). Cannabinoid receptor as a novel target for the treatment of prostate cancer. *Cancer Res.,* **65,** 1635–1641.

SARAFIAN, T.A., MAGALLANES, J.A., SHAU, H., TASHKIN, D. & ROTH, M.D. (1999). Oxidative stress produced by marijuana smoke. An adverse effect enhanced by cannabinoids. *Am. J. Respir Cell Mol. Biol.,* **20,** 1286–1293.

SARAFIAN, T.A., TASHKIN, D.P. & ROTH, M.D. (2001). Marijuana smoke and delta(9)-tetrahydrocannabinol promote necrotic cell death but inhibit Fas-mediated apoptosis. *Toxicol. Appl. Pharmacol.,* **174,** 264–272.

SCHLICKER, E. & KATHMANN, M. (2001). Modulation of transmitter release *via* presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.,* **22,** 565–572 Review.

SHIBATA, R., SATO, K., PIMENTEL, D.R., TAKEMURA, Y., KIHARA, S., OHASHI, K., FUNAHASHI, T., OUCHI, N. & WALSH, K. (2005). Adiponectin protects against myocardial ischemia–reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat. Med.,* **11,** 1096–1103.

SIMON, W.E., PAHNKE, V.G. & HOLZEL, F. (1985). *In vitro* modulation of prolactin binding to human mammary carcinoma cells by steroid hormones and prolactin. *J. Clin. Endocrinol. Metab.,* **60,** 1243–1249.

SMART, D., GUNTHORPE, M.J., JERMAN, J.C., NASIR, S., GRAY, J., MUIR, A.I., CHAMBERS, J.K., RANDALL, A.D. & DAVIS, J.B. (2000). The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br. J. Pharmacol.,* **129,** 227–230.

SONG, Z.H. & ZHONG, M. (2000). CB1 cannabinoid receptor-mediated cell migration. *J. Pharmacol. Exp. Ther.,* **294,** 204–209.

SRIVASTAVA, M.D., SRIVASTAVA, B.I. & BROUHARD, B. (1998). Delta9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. *Immunopharmacology,* **40,** 179–185.

STAMEY, T.A., YANG, N., HAY, A.R., MCNEAL, J.E., FREIHA, F.S. & REDWINE, E. (1987). Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N. Engl. J. Med.,* **317,** 909–916.

STEINBACH, G., LYNCH, P.M., PHILLIPS, R.K., WALLACE, M.H., HAWK, E., GORDAN, G.B., WAKABAYASHI, N., SAUNDERS, B., SHEN, Y., FUJIMURA, T., SU, L., LEVIN, B., DUBOIS, HITTELMAN, W.H., ZIMMERMAN, S., SHERMAN, J.W. & KELLOFF, G. (2000). The effect of celecoxib, a cyclooxygenase-2 inhibitor in familial adenomatous polyposis. *N. Engl. J. Med.,* **342,** 1946–1952.

STINCHCOMB, A., CHALLAPALLI, P., HARRIS, K. & BROWE, J. (2001). *Optimization of in vitro experimental conditions for measuring the percutaneous absorption of Δ9-THC, cannabidiol, and WIN55,212-2,* Symposium on the Cannabinoids, p. 161. Burlington, Vermont: International Cannabinoid Research Society.

STRAIKER, A., STELLA, N., PIOMELLI, D., MACKIE, K., KARTEN, H.J. & MAGUIRE, G. (1999). Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. *Proc. Natl. Acad. Sci. U.S.A.,* **96,** 14565–14570.

SUGIURA, T., KONDO, S., SUKAGAWA, A., NAKANE, S., SHINODA, A., ITOH, K., YAMASHITA, A. & WAKU, K. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.,* **215,** 89–97.

SUGIURA, T., OKA, S., GOKOH, M., KISHIMOTO, S. & WAKU, K. (2004). New perspectives in the studies on endocannabinoid and cannabis: 2-arachidonoylglycerol as a possible novel mediator of inflammation. *J. Pharmacol. Sci.,* **96,** 367–375 Review.

TOMIDA, I., PERTWEE, R.G. & AZUARA-BLANCO, A. (2004). Cannabinoids and glaucoma. *Br. J. Ophthalmol.,* **88,** 708–713.

TRIFAN, O.C., DURHAM, W.F., SALAZAR, V.S., HORTON, J., LEVINE, B.D., ZWEIFEL, B.S., DAVIS, T.W. & MASFERRER, J.L. (2002). Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. *Cancer Res.,* **62,** 5778–5784.

TSUJII, M. & DUBOIS, R.N. (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell,* **83,** 493–501.

TSUJII, M., KAWANO, S. & DUBOIS, R.N. (1997). Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 3333–3336.

TSUJII, M., KAWANO, S., TSUJI, S., SAWAOKA, H., HORI, M. & DUBOIS, R.N. (1998). Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell,* **93,** 705–716.

VACCANI, A., MASSI, P., COLOMBO, A., RUBINO, T. & PAROLARO, D. (2005). Cannabidiol inhibits human glioma cell migration through a cannabinoid receptor-independent mechanism. *Br. J. Pharmacol.,* **144,** 1032–1036.

VALK, PJ., HOL, S., VANKAN, Y., IHLE, J.N., ASKEW, D., JENKINS, N.A., GILBERT, D.J., COPELAND, N.G., DE BOTH, N.J., LOWENBERG, B. & DELWEL, R. (1997). The genes encoding the peripheral cannabinoid receptor and alpha-L-fucosidase are located near a newly identified common virus integration site, Evi11. *J. Virol.,* **71,** 6796–6804.

WALTER, L., FRANKLIN, A., WITTING, A., WADE, C., XIE, Y., KUNOS, G., MACKIE, K. & STELLA, N. (2003). Non-psychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.,* **23,** 1398–1405.

WARE, J.L. (1993). Growth factors and their receptors as determinants in the proliferation and metastasis of human prostate cancer. *Cancer Metast. Rev.,* **12,** 287–301.

WU, X., RUBIN, M., FAN, Z., DEBLASIO, T., SOOS, T., KOFF, A. & MENDELSOHN, J. (1996). Involvement of p27KiP1 in G1 arrest mediated by an antiepidermal growth factor receptor monoclonal antibody. *Oncogene,* **12,** 1397–1403.

YE, D., MENDELSOHN, J. & FAN, Z. (1999). Androgen and epidermal growth factor down-regulate cyclin-dependent kinase inhibitor p27KiP1 and costimulate proliferation of MDA PCa 2a and MDA PCa 2b prostate cancer cells. *Clin. Cancer Res.,* **5,** 2171–2177.

ZHA, S., YEGNASUBRAMANIAN, V., NELSON, W.G., ISAACS, W.B. & DE MARZO, A.M. (2004). Cyclooxygenases in cancer: progress and perspective. *Cancer Lett.,* **215,** 1–20 Review.

ZHU, L.X., SHARMA, S., STOLINA, M., GARDNER, B., ROTH, M.D., TASHKIN, D.P. & DUBINETT, S.M. (2000). Delta-9-tetrahydrocannabinol inhibits antitumor immunity by a CB2 receptor-mediated, cytokine-dependent pathway. *J. Immunol.,* **165,** 373–380.

ZYGMUNT, P.M., JULIUS, I., DI MARZO, I. & HOGESTATT, E.D. (2000). Anandamide – the other side of the coin. *Trends Pharmacol. Sci.,* **21,** 43–44.