Down-regulation of tissue inhibitor of metalloproteinases-1 in gliomas: a new marker of cannabinoid antitumoral activity?

Cristina Blázquez a, Arkaitz Carracedo a, María Salazar a, Mar Lorente a, Ainara Egia a, Luis González-Feria b, Amador Haro a, Guillermo Velasco a, Manuel Guzmán a,⁎

a Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, 28040 Madrid, Spain
b Department of Neurosurgery, Hospital Universitario de Canarias, La Laguna, 38320 Tenerife, Spain

Received 29 March 2007; received in revised form 23 May 2007; accepted 19 June 2007

Abstract

Cannabinoids, the active components of Cannabis sativa L. and their derivatives, inhibit tumor growth in laboratory animals by inducing apoptosis of tumor cells and inhibiting tumor angiogenesis. It has also been reported that cannabinoids inhibit tumor cell invasiveness, but the molecular targets of this cannabinoid action remain elusive. Here we evaluated the effects of cannabinoids on the expression of tissue inhibitors of metalloproteinases (TIMPs), which play critical roles in the acquisition of migrating and invasive capacities by tumor cells. Local administration of Δ⁹-tetrahydrocannabinol (THC), the major active ingredient of cannabis, down-regulated TIMP-1 expression in mice bearing subcutaneous gliomas, as determined by Western blot and immunofluorescence analyses. This cannabinoid-induced inhibition of TIMP-1 expression in gliomas (i) was mimicked by JWH-133, a selective CB₂ cannabinoid receptor agonist that is devoid of psychoactive side effects, (ii) was abrogated by fumonisin B₁, a selective inhibitor of ceramide synthesis de novo, and (iii) was also evident in two patients with recurrent glioblastoma multiforme (grade IV astrocytoma). THC also depressed TIMP-1 expression in cultures of various human glioma cell lines as well as in primary tumor cells obtained from a glioblastoma multiforme patient. This action was prevented by pharmacological blockade of ceramide biosynthesis and by knocking-down the expression of the stress protein p8. As TIMP-1 up-regulation is associated with high malignancy and negative prognosis of numerous cancers, TIMP-1 down-regulation may be a hallmark of cannabinoid-induced inhibition of glioma progression. © 2007 Elsevier Ltd. All rights reserved.

Keywords: Cannabinoid; Tissue inhibitor of metalloproteinases; Glioma; Invasion; Ceramide; Experimental therapeutics

1. Introduction

Cannabinoids, the active components of Cannabis sativa L. (marijuana) and their derivatives, exert a wide array of effects by activating specific receptors that are normally engaged by a family of endogenous ligands — the endocannabinoids (Howlett et al., 2002; Piomelli, 2003). Cannabis preparations have been used in medicine for centuries, and nowadays there is a renaissance in the study of their therapeutic effects (Di Marzo and Petrocellis, 2006; Mackie, 2006). Specifically, cannabinoids have been known to exert palliative effects in cancer patients since the early 1970s. The best established of these effects is the inhibition of chemotherapy-induced nausea and vomiting, and nowadays capsules of Δ⁹-tetrahydrocannabinol (THC), the major active component of cannabis, and its synthetic analogue nabilone are approved for that purpose (Guzmán, 2003; Hall et al., 2005). In addition, several clinical trials are testing other potential palliative properties of cannabinoids in oncology such as appetite stimulation and pain inhibition (Guzmán, 2003; Hall et al., 2005). Besides these palliative actions, cannabinoids have been proposed as potential antitumoral agents owing to their ability to inhibit the growth and angiogenesis of various types of tumor xenografts in animal models (Guzmán, 2003). Studies on malignant brain tumors (gliomas) and other models of cancer strongly support that cannabinoids decrease tumor progression by at least two mechanisms: the apoptotic death

* Corresponding author. Tel.: +34 913 944 668; fax: +34 913 944 672.
E-mail address: mgp@bbml.ucm.es (M. Guzmán).
of tumor cells (Galve-Roperh et al., 2000; Casanova et al., 2003; Carracedo et al., 2006a,b) and the inhibition of tumor angiogenesis (Blázquez et al., 2003, 2004, 2006; Casanova et al., 2003; Portella et al., 2003; Pisanti et al., 2007). It has also been reported that cannabinoids inhibit the migration and spreading of tumor cells (Portella et al., 2003; Blázquez et al., 2006; Grimaldi et al., 2006). However, the molecular targets of this cannabinoid effect remain elusive. Among the various factors involved in the acquisition of migrating and invasive capacities by cancer cells, the concerted action of matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases — TIMPs) plays a pivotal role. MMPs have been long linked to cancer cell invasion owing to their crucial involvement in extracellular matrix breakdown. Accordingly, increased expression and activation of MMPs is found in almost all human cancers compared with normal tissue, and this increase has been associated with poor patient prognosis (Egeblad and Werb, 2002; Deryugina and Quigley, 2006; Overall and Kleinfeld, 2006). Likewise, cannabinoid administration down-regulates MMP-2 expression in vascular endothelial and tumor cells (Blázquez et al., 2003; Pisanti et al., 2007). In addition to the MMPs, one of the most prominent MMP inhibitors, TIMP-1, is selectively up-regulated in numerous cancers, and this has been shown to be closely associated with negative prognosis, making TIMP-1 a promising candidate for new negative-progression marker (Hornebeck et al., 2005; Würtz et al., 2005; Yasui et al., 2005; Chirco et al., 2006). This finding may be explained, at least in part, by MMP-independent actions of TIMP-1 such as promotion of tumor cell proliferation and survival as well as of tumor angiogenesis (Hornebeck et al., 2005; Chirco et al., 2006). This background prompted us to explore the effect of cannabinoid administration on TIMP-1 expression by cancer cells. Here we report that cannabinoid administration inhibits TIMP-1 expression in cultured glioma cells, in mice bearing gliomas and in two patients with glioblastoma multiforme. In addition, our data support that this effect is mediated by the sphingolipid ceramide and the stress protein p8, two key signaling elements of cannabinoid antitumoral action (Guzmán, 2003; Blázquez et al., 2004; Carracedo et al., 2006a,b).

2. Methods

2.1. Cannabinoids

THC and JWH-133 were kindly given by Alfredo Dupetit (The Health Concept, Richelbach, Germany) and J.W. Huffman (Department of Chemistry, Clemson University, SC), respectively. For in vitro incubations cannabinoids were directly applied at a final DMSO concentration of 0.1—0.2% (v/v). For in vivo administration to mice cannabinoids were prepared at 1% (v/v) DMSO in 100 μl PBS supplemented with 5 mg/ml bovine serum albumin. No significant influence of the vehicle was observed on any of the parameters determined. The preparation of THC for administration to patients is described below.

2.2. Cell culture

The rat C6.9 and C6.4 glioma cell lines were cultured in Ham’s F12 medium supplemented with 10% fetal calf serum. The human SW1088, T98 G, U87 MG and U118 MG astrocytoma cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum. Twenty-four hours before the experiments, cells were transferred to their respective serum-free DMEM media. Primary human glioma cells were prepared from a glioblastoma multiforme (=grade IV astrocytoma). The biopsy was digested with collagenase (type IA; Sigma, St. Louis, MO) in DMEM at 37 °C for 90 min and the supernatant was seeded in DMEM containing 15% fetal calf serum and 1 mM glutamine. Cells were kept in primary culture for about 2 weeks, subsequently seeded for the experiments, and finally transferred to 0.5%-serum DMEM 24 h before cannabinoid addition. Cell viability was determined by the MTT test.

2.3. Tumor generation in mice

Tumors were generated in immune-deficient mice by subcutaneous flank inoculation of 5 × 10⁶ C6.9 or C6.4 glioma cells in 100 μl PBS supplemented with 0.1% glucose. When tumors had reached a volume of 300—400 mm³, animals were assigned randomly to the various groups and injected peritumorally (at approximately 2 mm from the tumor) for 8 days with 500 μg/day THC, 50 μg/day JWH-133 and/or 60 μg/day fumonisin B1 (Alexis, San Diego, CA). Control animals were injected with vehicle. Tumors were measured with external caliper and volume was calculated as (4π/3) × (width/2)² × length.

2.4. Human tumor samples

Tumor biopsies were obtained from two recurrent glioblastoma multiforme patients who had been treated with THC. The characteristics of the patients and the clinical study have been described in detail elsewhere (Blázquez et al., 2004; Guzmán et al., 2006). Briefly, each day an aliquot of THC (100 mg/ml in ethanol) was dissolved in 30 ml of physiological saline solution supplemented with 0.5% (w/v) human serum albumin and the resulting solution was administered intratumorally to the patients. Patient 1 received a total of 1.46 mg of THC for 30 days, while Patient 2 received a total of 1.29 mg of THC for 26 days (Guzmán et al., 2006). Samples were either frozen (for Western blotting) or fixed in formalin and embedded in paraffin (for immunomicroscopy).

2.5. Western blot analysis

Particulate tissue fractions were subjected to SDS-PAGE, and proteins were transferred from the gels onto polyvinylidene fluoride-dipore membranes. The blots were incubated with antibodies raised against TIMP-1 (1:200; Chemicon, Temecula, CA), TIMP-2 (1:1000, Chemicon) or TIMP-3 (1:1000; Affinity BioReagents, Golden, CO). α-Tubulin (1:4000, Sigma) was used as a loading control. In all cases, samples were subjected to luminography with an enhanced chemiluminescence detection kit (Amersham Life Sciences, Arlington Heights, IL). Densitometric analysis of the blots was performed with Kodak Molecular Imaging Software 4.0 in a Kodak Image Station 4000 MM (Rochester, NY).

2.6. Immunofluorescence microscopy

Mouse tumors were dissected and frozen, and sections were fixed in acetone for 10 min. Human tumors were fixed in 10%-buffered formalin and then paraffin-embedded. Sections (5 μm) were stained with anti-TIMP-1 antibody (1:50; Chemicon) as described previously (Blázquez et al., 2004). Sections were mounted with Mowiol mounting medium (Merck, Darmstadt, Germany) containing TOTO-3 iodide (1:1000; Molecular Probes, Leyden, The Netherlands) to stain cell nuclei. Fluorescence images were acquired using Meta- morph-Offline 6.2 software (Universal Imaging, Downingtown, PA) and Zeiss Axioplan 2 Microscope. Data were obtained from the analysis of five to 10 fields chosen randomly from three to four sections per tumor. Cells were routinely counted by an observer blinded to the experimental protocol, and positive cells were identified as green-stained cells with fluorescence thresholds set at 170 (low threshold) and 255 (high threshold).
2.7. Real-time quantitative PCR

Total RNA was prepared using TRIZOL reagent (Invitrogen, Carlsbad, CA). The generated cDNA was amplified by using the Universal Probe Library (Roche, Basel, Switzerland; Taqman probes 76F and 76R; primers for rat TIMP-1 5'-GCAAAGGCCTTCGAAAGA-3' and 5'-ATGGCTGAACGG GAAACAC-3'; primers for human TIMP-1 5'-GGGCTCACCAAGACCTA CA-3' and 5'-TGCAAGGGATGGAATAACAG-3'). Each value was adjusted by calculating its ratio to 18S RNA levels, which were determined by using specific multispecies primers (Roche; Taqman probes 55F and 55R; primers 5'-AAATCGATTTATGGTTCCTTTGGTC-3' and 5'-CTTGGTCAGTTATCCAA-3'). Amplifications were run in a 7900 Real-Time PCR System (Applied Biosystems, Foster City, CA) (Carracedo et al., 2006b).

2.8. RNA interference

U87 MG human astrocytoma cells were transfected with siRNA duplexes using the X-tremeGENE siRNA Transfection Reagent (Roche) as described before (Carracedo et al., 2006b). Double-stranded RNA duplexes corresponding to human p8 (5'-GGAGGACCCAGGACAGGAU-3') and a non-targeted control (5'-UUCUCCGAACGUGUCACGU-3') were purchased from Eurogentec (Liege, Belgium). RT-and real-time qualitative PCR analyses of p8 and GAPDH mRNA were performed as described (Carracedo et al., 2006a,b).

2.9. Cell migration assays

Cell migration was monitored by using Boyden chambers. After exposure to cannabinoids for 24 h, cells were trypsinized, washed, resuspended in DMEM and loaded onto the upper chamber well. DMEM supplemented with 2.5% FBS was placed in the lower chamber well as a cell-migration stimulant. Cells were allowed to migrate for 4 h (C6.9 and C6.4 cells) or 6 h (U87 MG cells) at 37°C. Cells were allowed to migrate for 4 h (C6.9 and C6.4 cells) or 6 h (U87 MG cells) at 37°C. Trypsinized, washed, and resuspended in DMEM, the remaining cells on the bottom side of the membrane were fixed with 70% ethanol, stained with crystal violet and counted.

2.10. Statistics

Results shown represent mean ± S.D. Statistical analysis was performed by ANOVA with a post hoc analysis by the Student–Neuman–Keuls test or by unpaired Student t-test.

3. Results

3.1. THC inhibits TIMP-1 expression in mouse gliomas

To test whether cannabinoid administration affects TIMP-1 levels, we generated tumors by subcutaneous inoculation of glioma cells to mice. The specificity of cannabinoid action was ascertained by the parallel study of C6.9 and C6.4 glioma cells, which constitute well established models of cannabinoid-responsive and cannabinoid-resistant cells, respectively (Galve-Roperh et al., 2000; Gómez del Pulgar et al., 2002; Carracedo et al., 2006b). Tumors were treated with either vehicle or THC and TIMP-1 levels determined by Western blot analysis. Cannabinoid administration decreased tumor growth and TIMP-1 expression in C6.9-cell gliomas (Fig. 1a and b). This effect was TIMP family member-selective as THC treatment did not affect TIMP-2 and TIMP-3 expression in the tumors (Fig. 1b). In contrast to C6.9-cell gliomas, neither tumor growth nor TIMP-1 expression was affected by THC treatment in C6.4-cell tumors (Fig. 1a and c). Immunofluorescence microscopy experiments confirmed the decrease of TIMP-1 immunoreactivity upon cannabinoid administration in the cannabinoid-sensitive but not in the cannabinoid-resistant tumors (Fig. 2).

3.2. THC inhibits TIMP-1 expression in two glioblastoma multiforme tumor models

To obtain further support for the potential therapeutic implication of cannabinoid-induced down-regulation of TIMP-1 expression, we analyzed tumor samples from two patients enrolled in a clinical trial aimed at investigating the effect of THC on recurrent glioblastoma multiforme (Guzmán et al., 2006). The patients were subjected to local THC administration and biopsies were taken before and after treatment. Immunocytochemistry analyses showed that tumor TIMP-1 levels were lower after cannabinoid delivery in both patients (Fig. 3a). This was confirmed by Western blot analysis in tumor biopsies from Patient 1, in which TIMP-2 and TIMP-3 levels remained unchanged (Fig. 3b). Unfortunately, we were unable to obtain appropriate samples for Western blotting from Patient 2.

3.3. The CB2 receptor agonist JWH-133 inhibits TIMP-1 expression in mouse gliomas

Because cannabinoid-based therapeutic strategies should be as devoid as possible of psychotropic effects, which are mediated by brain CB1 receptors (Piomelli, 2003), and glioma express CB2 receptors (Galve-Roperh et al., 2000), which are not linked to cannabinoid psychoactivity, we administered to mice JWH-133, a CB2 receptor-selective agonist that exerts antitumoral activity without overt psychoactive side effects (Sánchez et al., 2001). JWH-133 decreased tumor growth (Fig. 4a) as well as TIMP-1 expression in the remaining glioma xenografts, as determined by both Western blot (Fig. 4b) and immunocytochemistry analyses (Fig. 4c). TIMP-2 and TIMP-3 levels remained unchanged upon JWH-133 treatment (Fig. 4b).

3.4. The ceramide biosynthesis inhibitor fumonisin B1 prevents cannabinoid-induced TIMP-1 down-regulation in mouse gliomas

The sphingolipid messenger ceramide has been implicated in the regulation of tumor growth and angiogenesis by cannabinoids (Galve-Roperh et al., 2000; Gómez del Pulgar et al., 2002; Blázquez et al., 2004; Carracedo et al., 2006b). The involvement of ceramide in the inhibition of TIMP-1 expression by cannabinoids was thus tested by the use of fumonisin B1, a selective inhibitor of ceramide synthesis de novo that prevents cannabinoid-induced ceramide accumulation in glioma cells (Blázquez et al., 2004). Fumonisin B1 abrogated the inhibitory action of JWH-133 on tumor growth (Fig. 4a). In addition, the decrease in TIMP-1 levels induced by cannabinoid administration to mice was prevented by co-treatment of the animals with...
3.5. THC inhibits TIMP-1 expression in cultured glioma cells via ceramide and p8

To test whether the cannabinoid-induced down-regulation of TIMP-1 expression observed in vivo reflects the direct impact of cannabinoids on tumor cells, we conducted experiments in cultured glioma cells. In line with the aforementioned in vivo observations, THC decreased TIMP-1 mRNA in cultures of the sensitive C6.9 glioma cell line but not of the resistant C6.4 cells (Fig. 5a). The cannabinoid did not affect cell viability throughout the time interval in which TIMP-1 determinations were performed (up to 24 h, data not shown), and thus changes in TIMP-1 expression preceded cannabinoid-evoked apoptosis. Cannabinoid-induced inhibition of TIMP-1 expression was also evident in a number of human fumonisin B1, as determined by both Western blot (Fig. 4b) and immunomicroscopy analyses (Fig. 4c).

Fig. 1. THC inhibits TIMP-1 expression in mouse gliomas as determined by Western blot. Mice bearing tumors generated by inoculation of C6.9 or C6.4 glioma cells were treated with either vehicle or THC for 8 days as described in Section 2. (a) Tumor volume at the end of the treatment. (b,c) TIMP-1, TIMP-2 and/or TIMP-3 expression as determined by Western blot. Optical density values relative to those of α-tubulin are given in arbitrary units. Significantly different (**P < 0.01) from control animals (n = 3–4 for each experimental group).

Fig. 2. THC inhibits TIMP-1 expression in mouse gliomas as determined by immunofluorescence microscopy. Mice bearing tumors generated by inoculation of C6.9 or C6.4 glioma cells were treated with either vehicle or THC for 8 days as described in Section 2 and TIMP-1 expression was determined by immunofluorescence microscopy (green). Cell nuclei are stained in blue. One representative tumor of each experimental group is shown. Quantification of the percentage of TIMP-1-positive cells over total cells is given. Significantly different (**P < 0.01) from control animals (n = 3–4 for each experimental group).
glioma cell lines (astrocytomas SW1088, T98 G, U87 MG and U118 MG) and, more importantly, in tumor cells directly obtained from a human glioblastoma multiforme biopsy (Fig. 5b). The cannabinoid effect was prevented by ISP-1 (Fig. 5c), a selective inhibitor of ceramide synthesis de novo that blocks THC-induced ceramide accumulation in glioma cells (Carracedo et al., 2006b).

We have previously reported that the stress protein p8 is a ceramide effector in cannabinoid proapoptotic and antitumoral actions (Carracedo et al., 2006b). To determine the role of p8 in THC-induced down-regulation of TIMP-1, we selectively reduced p8 expression by RNA interference. As shown in Fig. 5d, knock-down of p8 mRNA prevented the inhibitory action of THC on TIMP-1 expression.

3.6. THC inhibits the migration of cultured glioma cells

TIMP-1 modulates tumor cell migration and invasion. Most of the classical studies on this issue have reported that TIMP-1 overexpression inhibits invasion and migration of tumor (including glioma) cells (Van Meter et al., 2001; Egeblad and Werb, 2002; Rao, 2003). However, in more recent studies TIMP-1 has been shown to stimulate several mitogenic signal pathways (e.g. the phosphatidylinositol 3-kinase/Akt and Ras/extracellular signal-regulated kinase pathways) usually linked to enhanced cell invasion and migration (Hornebeck et al., 2005; Chirco et al., 2006). We therefore examined whether cannabinoids affect glioma cell migration. As shown in Fig. 6, THC inhibited the migration of the sensitive C6.9 glioma and U87 MG astrocytoma cell lines, but not of the resistant C6.4 cell line.

4. Discussion

Despite the widely described antitumoral actions of cannabinoids in various animal models of cancer (Guzmán, 2003), the molecular effectors of these actions have not been fully characterized as yet. Here we show that cannabinoid administration down-regulates TIMP-1 expression in mice bearing gliomas as well as in two patients with recurrent glioblastoma multiforme. Cannabinoid-induced inhibition of TIMP-1 expression was also evident in cultured glioma cells, indicating that the changes observed in vivo reflect — at least in part — the direct impact of cannabinoids on tumor cells. TIMP-1 expression is selectively up-regulated in numerous cancers, including breast cancer, gastric cancer, colorectal cancer, lymphoma and non-small cell lung carcinoma, and this has been shown to be closely associated with negative prognosis, making TIMP-1 a promising candidate for new poor-prognosis marker (Hornebeck et al., 2005; Würtz et al., 2005; Yasui et al., 2005; Chirco et al., 2006). These findings were initially unexpected considering the well established role of TIMP-1 in the inhibition on MMP-mediated extracellular matrix degradation and tissue invasion by tumor cells (Egeblad and Werb, 2002; Rao, 2003). Nowadays, however, those observations
may be explained by newly discovered MMP-independent actions of TIMP-1 such as promotion of tumor cell proliferation and survival as well as of tumor angiogenesis (Hornebeck et al., 2005; Chirco et al., 2006). In fact, when TIMP-1 was initially characterized about 20 years ago, it was found to be identical to the erythroid-potentiating activity factor that was able to stimulate the growth and survival of a wide range of transformed and non-transformed cells (Docherty et al., 1985). Moreover, accruing basic and clinical evidence supports that the relationship between MMPs and cancer is very far from simple as MMPs can either enhance or inhibit tumor progression depending on factors such as the type of MMP and tumor and the characteristics of the experimental setting (Folgueras et al., 2004; Overall and Kleifeld, 2006; Strongin, 2006). Within this very complex scenario of MMP and TIMP actions on tumor progression, it is becoming nonetheless clear that TIMP-1 can be a marker of high tumor malignancy and poor tumor prognosis, which fits well with its down-regulation by cannabinoid compounds reported here. We are nonetheless aware that further work is necessary to determine whether cannabinoid-induced inhibition of TIMP-1 expression has a causative role in cannabinoid antitumoral actions such as the inhibition of tumor cell migration (present report), the induction of tumor cell death and the inhibition of tumor angiogenesis.

It has been shown that cannabinoids modulate sphingolipid-metabolizing pathways, thereby increasing the intracellular
levels of ceramide (Galve-Roperh et al., 2000; Guzmán, 2003), a lipid second messenger that inhibits cell growth and survival in different systems (Ogretmen and Hannun, 2004). Specifically, the stimulation of ceramide synthesis de novo is critically involved in cannabinoid-induced apoptosis of glioma cells (Gómez del Pulgar et al., 2002; Carracedo et al., 2006b) and inhibition of glioma angiogenesis (Blázquez et al., 2004). The findings reported here expand the role of de novo-synthesized ceramide in cannabinoid antitumoral action and support that this lipid messenger is involved in the regulation of TIMP-1 expression. In the context of the “sphingolipid rheostat” theory, the antiproliferative sphingolipid ceramide would blunt TIMP-1 expression (present study) and tumor growth (Galve-Roperh et al., 2000), whereas the mitogenic sphingolipid sphingosine 1-phosphate would shift the balance towards TIMP-1 up-regulation (Yamanaka et al., 2004) and tumorogenesis (Ogretmen and Hannun, 2004).

The use of cannabinoids in medicine is limited by the psychoactive effects mediated by neuronal CB1 receptors (Howlett et al., 2002; Piomelli, 2003). Although these adverse effects are within the range of those accepted for other medications, especially 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 (Guzmán, 2003; Di Marzo and Petrocellis, 2006; Mackie, 2006). As glioma cells express functional CB2 receptors (Galve-Roperh et al., 2000), we used a non-psychoactive, CB2 receptor-selective ligand to depress TIMP-1 expression. Administration of selective CB2 receptor agonists to mice has also been shown to inhibit the growth and angiogenesis of gliomas (Sánchez et al., 2001; Blázquez et al., 2003, 2004), skin carcinomas (Casanova et al., 2003) and melanomas (Blázquez et al., 2006), supporting the possibility of finding cannabinoid-based

Fig. 5. THC inhibits TIMP-1 expression in cultured glioma cells via ceramide and p8. (a) C6.9 and C6.4 glioma cells were cultured for 24 h with vehicle (open bars) or 1.5 μM THC (closed bars) and TIMP-1 expression was determined (n = 4). (b) Primary tumor cells obtained from a patient with glioblastoma multiforme (GBM), SW1088, T98 G, U87 MG and U118 MG astrocytoma cells were cultured for 24 h with vehicle (open bars), 7 μM THC (GBM) or 1.5 μM THC (SW1088, T98 G, U87 MG and U118 MG) (closed bars) and TIMP-1 expression was determined (n = 2). (c) U87 MG astrocytoma cells were cultured for 24 h with vehicle, 1.5 μM THC, 1.5 μM ISP-1 or 1.5 μM THC plus 1.5 μM ISP-1 and TIMP-1 expression was determined (n = 3). (d) U87 MG astrocytoma cells were transfected with a control (C) or a p8-directed siRNA and were cultured for 24 h with vehicle (open bars) or 1.5 μM THC (closed bars). Finally, TIMP-1 expression was determined (n = 3). Inset: representative RT-PCR experiment showing p8 mRNA knock-down by siRNA-p8 (relative values of p8 mRNA expression as determined by real-time quantitative PCR are given). In all panels, data represent values of TIMP-1 mRNA levels as determined by real-time quantitative PCR. Significantly different (**P < 0.01, *P < 0.05) from vehicle incubations.
References


Acknowledgements

We are indebted to Eva Resel for expert technical assistance and to the rest of the members of the lab for discussion and advice. This work was supported by grants from Ministerio de Educación y Ciencia (SAF2006-00918 to M.G.), Comunidad de Madrid (S-SAL/0261/2006 to M.G.) and Universidad Complutense de Madrid (PR1/07-14896 to C.B.).

Fig. 6. THC inhibits the migration of cultured glioma cells. C6.9 and C6.4 glioma cells, as well as U87 MG astrocytoma cells, were cultured for 24 h with vehicle (open bars) or 1.5 μM THC (closed bars) and cell migration was determined as described in Section 2 (n = 4). Significantly different (**P < 0.01) from vehicle incubations.

antitumoral strategies devoid of non-desired psychotropic side effects.


