Cannabinoids Induce Apoptosis of Pancreatic Tumor Cells via Endoplasmic Reticulum Stress–Related Genes

Arkaitz Carracedo,1 Meritxell Gironella,2 Mar Lorente,1 Stephane Garcia,2 Manuel Guzmán,1 Guillermo Velasco,1 and Juan I. Iovanna1

1Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, Madrid, Spain and 2U624 Institut National de la Sante et de la Recherche Medicale, Marseille, France

Abstract

Pancreatic adenocarcinomas are among the most malignant forms of cancer and, therefore, it is of especial interest to set new strategies aimed at improving the prognostic of this deadly disease. The present study was undertaken to investigate the action of cannabinoids, a new family of potential antitumoral agents, in pancreatic cancer. We show that cannabinoid receptors are expressed in human pancreatic tumor cell lines and tumor biopsies at much higher levels than in normal pancreatic tissue. Studies conducted with MiaPaCa2 and Panc1 cell lines showed that cannabinoid administration (a) induced apoptosis, (b) increased ceramide levels, and (c) up-regulated mRNA levels of the stress protein p8. These effects were prevented by blockade of the CB2 cannabinoid receptor or by pharmacologic inhibition of ceramide synthesis de novo. Knockdown experiments using selective small interfering RNAs showed the involvement of p8 via its downstream endoplasmic reticulum stress–related targets activating transcription factor 4 (ATF-4) and TRB3 in Δ9-tetrahydrocannabinol–induced apoptosis. Cannabinoids also reduced the growth of tumor cells in two animal models of pancreatic cancer. In addition, cannabinoid treatment inhibited the spreading of pancreatic tumor cells. Moreover, cannabinoid administration selectively increased apoptosis and TRB3 expression in pancreatic tumor cells but not in normal tissue. In conclusion, results presented here show that cannabinoids lead to apoptosis of pancreatic tumor cells via a CB2 receptor and de novo synthesized ceramide-dependent up-regulation of p8 and the endoplasmic reticulum stress–related genes ATF-4 and TRB3. These findings may contribute to set the basis for a new therapeutic approach for the treatment of pancreatic cancer.

Introduction

Pancreatic cancer is one of the most malignant and aggressive forms of cancer (1). With an incidence of 10/10,000 for men and 7/10,000 for women, it represents the fourth most common death-causing cancer in the United States (2) and the fifth in the Western world overall (3). About 95% of pancreatic cancers cases are ductal adenocarcinomas. The anatomic localization of the pancreas and the nonspecific nature of the symptoms result in a complex and delayed diagnosis. Therefore, at the time of detection, 85% of patients show metastatic infiltrations in proximal lymphatic nodes, liver, or lungs, and only 15% to 20% of the tumors are typically found resectable (1). In addition, <20% of the operated patients survive up to 5 years. Treatment of unresectable tumors is currently based on administration of fluorouracil chemoradiation for locally advanced tumors and gemcitabine chemotherapy for metastatic disease (1). However, despite maximal optimization of these therapies, the median survival for the affected patients remains ~1 year. It is therefore of especial interest to set new therapeutic strategies aimed at improving the prognostic of this deadly disease.

The hemp plant Cannabis sativa produces ~70 unique compounds known as cannabinoids, of which Δ9-tetrahydrocannabinol (THC) is the most important owing to its high potency and abundance in cannabis (4). THC exerts a wide variety of biological effects by mimicking endogenous substances, the endocannabinoids anandamide (5) and 2-arachidonoylglycerol (6), which bind to and activate specific cannabinoid receptors. Thus far, two cannabinoid-specific G protein-coupled receptors have been cloned and characterized from mammalian tissues (7): The CB1 receptor is particularly abundant in discrete areas of the brain but is also expressed in peripheral nerve terminals and various extraneuronal sites. In contrast, the CB2 receptor was initially described to be present in the immune system (8) although, recently, it has been shown that expression of this receptor also occurs in cells from other origins (9–11).

One of the most exciting areas of research in the cannabinoid field is the study of the potential application of cannabinoids as antitumoral agents (12). Thus, cannabinoid administration has been shown to curb the growth of several models of tumor xenografts in rats and mice (12). This antitumoral action of cannabinoids relies, at least in part, on the ability of these compounds to directly affect the viability, via induction of apoptosis or cell cycle arrest, of a wide spectrum of tumor cells in culture (12). In addition, cannabinoid treatment inhibits tumor angiogenesis (13–15). Both CB1 (9, 11, 16, 17) and CB2
receptors (11, 18, 19) have been shown to mediate the growth-inhibiting action of THC and related cannabinoids on tumor cells (12). The present study was therefore undertaken to investigate (a) the antitumoral action of cannabinoids in pancreatic cancer and (b) the molecular mechanisms involved in that effect.

Materials and Methods

Reagents. THC was a kind gift from Dr. Javier Fernández-Ruiz (Complutense University, Madrid, Spain) and SR141716 and SR144528 were a kind gift from Sanofi Synthelabo (Montpellier, France). WIN 55,212-2 was from Sigma Chemical Co. (St. Louis, MO), myriocin (ISP-1) was from Biomol (Plymouth Meeting, PA), and C2-ceramide was from Alexis (San Diego, CA).

Cell culture and viability. MiaPaCa2 and Panc1 were cultured in DMEM containing 10% heat-inactivated fetal bovine serum (FBS), 5 units/mL penicillin, and 5 mg/mL streptomycin. Capan2 and BxPc3 were cultured in RPMI 1640 containing 10% heat-inactivated FBS, 2 mmol/L glutamine, 5 units/mL penicillin, and 5 mg/mL streptomycin. Cells were transferred to a serum-free medium (except Capan2 and BxPc3, which were transferred to a medium containing 0.1% serum) 18 hours before the different treatments. THC, WIN 55,212-2, SR141716, and SR144528 stock solutions were prepared in DMSO. Control incubations contained the same amount of DMSO. No significant effect of DMSO was observed in any of the parameters determined throughout this study at the final concentration used (0.1-0.2%, v/v). Cell viability was determined using the CellTiter96 Aqueous One Solution Reagent (MTS, Promega, Madison, WI) according to the instructions of the manufacturer.

Transfections. Seventy-five percent confluent MiaPaCa2 cells were transfected with the different small interfering RNAs (siRNA) using the X-tremeGENE siRNA Transfection Reagent (Roche, Basel, Switzerland) according to the instructions of the manufacturer. Twenty-four hours after transfection, cells were trypsinized and seeded at a density of 5,000/cm². Cells were transferred to a serum-free medium 18 hours before the different treatments. Transfection efficiency was monitored using a control fluorescent siRNA (Qiagen, Hilden, Germany).

RNA interference. Double-stranded RNA duplexes corresponding to human p8 (5'-GGAGGAACCGAGAACAG) human activating transcription factor 4 (ATF-4: 5'-GCUAGGUCUCUUAUGAG), human TRB3 (5'-UCAUCUAAGAGAACCCG), and a nontargeted control (5'-UUCUCCGUGUGACGU) were purchased from Eurogentec (Liege, Belgium).

Caspase 3/7 activity. Caspase 3/7 (DVEDase activity) was determined according to the instructions of the manufacturer using a luminogenic substrate (Caspase Glo, Promega). Luminiscence was determined in a Microplate Fluorescence Reader FLUOstar Optima (BMG Labtech, Offenburg, Germany). One unit of caspase activity is defined as the amount of active enzyme necessary to produce an increase in the luminiscence of 1 arbitrary unit after 1 hour.

Figure 1. Pancreatic tumor cells express cannabinoid receptors. A, total RNA was isolated from the corresponding cell lines and reverse transcription-PCR (RT-PCR) using selective primers for human CB1, CB2, or GAPDH was done. Representative of two experiments. P, Panc1; M, MiaPaCa2; B, BxPc3; C, Capan2. B, cell lysates were obtained from the corresponding cell lines and CB1, CB2, and α-tubulin protein levels were determined by Western blot. Spleen (Sp) was used as a positive control for CB1 and CB2 expression. Representative of two experiments. C, representative (63×) images of CB receptor–stained pancreatic tumor cells. Cannabinoid receptors are stained in red; nuclei are stained in green. D, total RNA was isolated from pancreatic biopsies diagnosed as metastatic adenocarcinoma, adenocarcinoma, and normal pancreas, and CB1 and CB2 mRNA levels were determined by real-time quantitative PCR. The plot represents gene expression (in fold number) of each individual sample relative to one of the tumor samples. mRNA levels in normal pancreas samples were much lower (<1,000-fold (CB1 receptor) or <100,000-fold (CB2 receptor)) than in the tumor of reference. N.D., nondetectable. E, representative (63×) images of CB receptor immunostaining obtained from three human pancreatic tumor biopsies and three samples of normal human pancreas. Cannabinoid receptors are stained in green; nuclei are stained in blue.
Ceramide levels. Ceramide levels were determined as previously described (20). Briefly, after incubation of the cells in the different conditions, lipids were extracted, saponified, and incubated with E. coli diacylglycerol kinase in the presence of \( \gamma^{32}\text{P}\text{ATP} \). Finally, ceramide 1-phosphate was resolved by TLC.

Reverse transcription-PCR analysis. RNA was isolated using the RNeasy Protect kit (Qiagen) including a DNase digestion step using the RNase-free DNase kit (Qiagen). cDNA was subsequently obtained using the first-strand cDNA synthesis kit (Roche). The following sense and antisense primers, respectively, were used to amplify human CB1 (CGTGGGCAGCTGTCTGTCAGCA and CATGCGGGCTTGGTCTGG; 408-bp product), human CB2 (CGCCGGAGCTGCTCATACC and CCTCATTCGGGCCATTCCTG; 522-bp product), human p8 (GAAGAGAGGCAGGGAAGACA and CTGCCGTGGCTGTCTATTTA; 571-bp product), human TRB3 (GCCACTGCCTCCCGTCTTG and GCTGCCTTGCCCGAGTATGA; 538-bp product), human ATF-4 (AGTCGGGTTTGGGGGCTGAAG and TGGGGAAAGGGGAGGTTGTA; 436-bp product), and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GGGAAGCTCACTGGCATGGCCTTCC and CATGTGGGCCATGAGGTCCACCAC; 322-bp product). PCR reactions were done using the following parameters: 95°C for 5 minutes, 94°C for 30 seconds, 57°C (CB1, CB2, p8, ATF-4, and GAPDH) or 52°C (TRB3) for 30 seconds, and 72°C for 1 minute followed by a final extension step of 72°C for 5 minutes. The number of cycles (25-26 cycles for ATF-4, 26-28 cycles for p8, 28-29 cycles for TRB3, 22-25 cycles for GAPDH, and 36-38 cycles for CB1 and CB2) was adjusted to allow detection in the linear range. Finally, PCR products were separated on 1.5% agarose gels.

Real-time quantitative PCR. cDNA was obtained with Transcriptor (Roche). TaqMan probes were obtained from Applied Biosystems (Foster City, CA). Amplifications were run in a 7900 Real-time PCR System (Applied Biosystems). Each value was adjusted by using 18S RNA levels as reference.

Human tumor samples. Pancreatic tumor samples were obtained from “Hopital Nord,” Marseille, France.

S.c. xenografts. Tumors were induced by s.c. injection of \( 20 \times 10^6 \) MiaPaCa2 cells in PBS supplemented with 0.1% glucose in immunodeficient nude mice. When tumors reached an average size of 250 mm\(^3\), animals were assigned randomly to various groups and injected peritumorally for 15 days with THC (15 mg/kg/d), JWH (1.5 mg/kg/d), or vehicle in 100 \( \mu \)L of PBS supplemented with 5 mg/mL defatted and dialyzed bovine serum albumin (BSA). Tumors were measured with external caliper and volume was calculated as \( (4/3) \pi \times (width/2)^2 \times (length/2) \).

Intrapancreatic tumors. Immunodeficient mice were anesthetized, operated to allow access to the abdominal cavity, and \( 10 \times 10^6 \) MiaPaCa2 cells were injected directly in the pancreas. Animals were allowed to recover from the operation for 10 hours and assigned randomly to control or treatment groups. Vehicle or WIN 55,212-2 (in 150 \( \mu \)L of PBS supplemented with 5 mg/mL defatted and dialyzed BSA) was given daily for 14 days in a single i.p. injection (1.5 mg/kg for 2 days, 2.25 mg/kg for 2 additional days, and 3.0 mg/kg for 10 additional days).
Western blot. Western blot analysis was done as described (20). Anti-CB1 and anti-CB2 receptor antibodies (Affinity Bioreagents, Golden, CO) were used.

Immunostaining and terminal deoxyribonucleotidyl transferase-meditated dUTP nick end labeling. Pancreatic tumor cell lines were fixed in 4% buffered paraformaldehyde. Samples from human biopsies and tumor xenografts were dissected and fixed in 10% buffered formalin and then paraffin embedded. After deparaffinization, H&E-safran staining was done (samples from tumor xenografts). For the rest of experiments, samples were blocked to avoid nonspecific binding. Terminal deoxyribonucleotidyl transferase-meditated dUTP nick end labeling (TUNEL) was done using the in situ cell death detection kit (Roche). For CB receptors and TRB3 immunodetection, slides were incubated with a rabbit anti-human/mouse CB1 receptor antibody (1:500; Affinity Bioreagents), a rabbit anti-human/mouse CB2 receptor antibody (1:500; Affinity Bioreagents), and a rabbit anti-TRB3 antibody (1:500; Abcam, Cambridge, United Kingdom). Slides were further incubated in the dark (1 hour, room temperature) with a secondary antirabbit antibody-Alexa Fluor 594 (1:500; cell lines and tumor xenografts) or an antirabbit antibody-Alexa Fluor 488 (1:500; samples from human biopsies; Molecular Probes, Leyden, the Netherlands). Finally, Yoyo-1 (Molecular Probes; 1:5,000; cell lines and tumor xenografts) or Hoechst 33342 (Sigma Chemical; 10 μg/mL; human biopsies) were added to stain nuclei. Fluorescence images were acquired using a Leica Axiointert S100 TV microscope (Carl Zeiss, Oberkochen, Germany). Pixel quantification was determined with Metamorph-Offline software (Universal Imaging, Downingtown, PA).

Statistics. Unless otherwise specified, results shown represent mean ± SD Statistical analysis was done by ANOVA with a post hoc analysis by the Student-Neuman-Keuls test, except for data in Fig. 5E and F, which were analyzed by a χ² test.

Results

Activation of the CB2 cannabinoid receptor induces apoptosis of pancreatic tumor cells in vitro. We investigated the effect of cannabinoids on pancreatic tumor cells. First, we determined the expression of cannabinoid receptors in four different human pancreatic tumor cell lines as well as in biopsies of human pancreatic tumors. CB1 and CB2 cannabinoid receptor mRNA (Fig. 1A) and protein (Fig. 1B and C) were expressed in Panc1, MiaPaCa2, Capan2, and BxPc3 cell lines. In addition, mRNA
for cannabinoid receptors was expressed in several human pancreatic tumor biopsies, whereas in samples obtained from normal pancreatic tissue, mRNA levels for these receptors were very low or could not be detected (Fig. 1D). This difference between tumor and nontransformed pancreatic tissue was further confirmed by immunofluorescence analysis of CB receptors both in human biopsies from pancreatic cancer (Fig. 1E) and in pancreatic tumors generated in mice (Supplementary Fig. S1; see below). In both cases, expression of cannabinoid receptors was detected clearly in the tumor nodules but hardly in the surrounding pancreatic tissue.

Next, we tested the effect of THC on cell viability. Incubation with THC led to a dose-dependent decrease in cell viability in the four lines tested (Fig. 2A). Because cells exhibited a different sensitivity to THC treatment, we chose MiaPaCa2 as the most sensitive line and Panc1 as a less sensitive line to confirm the involvement of cannabinoid receptors in THC antiproliferative action. Incubation with the CB2-selective antagonist SR144528, but not with the CB1-selective antagonist SR141716, prevented THC-induced loss of cell viability in both lines (Fig. 2B and C). Likewise, in MiaPaCa2 and Panc1 cells, THC led to caspase-3 activation, a characteristic of apoptotic cell death, and preincubation with SR144528 abrogated this effect (Fig. 2D and E).

Because de novo synthesized ceramide has been implicated in CB receptor–mediated apoptosis of glioma cells (19, 20), we tested the involvement of this pathway in our model. As shown in Supplementary Fig. S2A, incubation with THC led to ceramide accumulation in MiaPaCa2 and Panc1 cell lines, and preincubation with SR144528 or ISP-1, a selective inhibitor of serine palmitoyltransferase (the enzyme that catalyzes the rate-limiting step of ceramide biosynthesis; ref. 21), prevented this accumulation. In addition, ISP-1 prevented the THC-induced (a) decrease of cell viability (Fig. 2B and C) and (b) activation of caspase 3 (Supplementary Fig. S2B and C), indicating that de novo synthesized ceramide is involved in THC-induced apoptosis of pancreatic tumor cells.

The stress-regulated protein p8 is involved in THC-induced apoptosis of pancreatic tumor cells. p8 (also designated as candidate of metastasis 1) is a stress-regulated protein related to the architectural factor HMG-I/Y (22). This protein has been implicated in a number of functions including the induction of apoptosis of pancreatic tumor cells (23). In addition, it has been shown that ceramide treatment leads to p8 up-regulation (24) and we have very recently identified this protein as an essential mediator of cannabinoid antitumoral action in gliomas (25). We therefore tested the involvement of this protein in the antiproliferative effect of THC in our cells. p8 mRNA levels increased after THC treatment of MiaPaCa2 cells, and incubation with SR144528 (Fig. 3A) or ISP-1 (Fig. 3B) prevented this effect. Moreover, knockdown of p8 mRNA using a selective siRNA prevented THC-induced apoptosis of MiaPaCa2 cells (Fig. 3C), confirming the implication of this gene in the response to THC in our model.

Our next step was to identify genes downstream of p8 that could participate in the antitumoral effect of THC. By comparing the mRNA expression profiles of p8-deficient fibroblasts and fibroblasts with enforced p8 expression, several p8-dependent genes have been implicated in apoptotic signaling (25). Among these genes, we selected the endoplasmic reticulum (ER) stress–related proteins ATF-4 (26, 27) and TRB3 (28) as potential mediators of p8-dependent effects in our cells.

Incubation with THC led to a parallel increase in p8, ATF-4, and TRB3 mRNA levels, which was prevented by incubation with ISP-1 (Fig. 3D). In addition, knockdown of p8 mRNA prevented ATF-4 and TRB3 up-regulation (Fig. 3E). Moreover, knockdown of ATF-4 or TRB3 mRNA also prevented THC-induced apoptosis (Fig. 3F). Taken together, these observations support that challenge with THC triggers a ceramide- and p8-controlled apoptotic response in pancreatic tumor cells, which involves up-regulation of these genes.

Antitumoral effect of cannabinoid in pancreatic cancer models in vivo. To evaluate the antiproliferative effect of cannabinoids on pancreatic tumors in vivo, we first generated tumor xenographs by s.c. injection of MiaPaCa2 cells in immuno-deficient mice. As shown in Fig. 4, peritumoral treatment with THC or the CB2-selective (and therefore psychoactivity devoid; ref. 19) cannabinoid agonist JWH-133 reduced notably the growth of the established pancreatic tumors.

Next, we generated tumors by intrapancreatic injection of MiaPaCa2 cells to investigate the antitumoral effect of cannabinoids in a model that resembles more closely the niche of pancreatic cancer spreading. The synthetic cannabinoid agonist WIN 55,212-2 was selected for ip. administration owing to its better bioavailability than THC and other classic cannabinoids. Experiments were done to confirm that this compound, which exhibits affinity for both cannabinoid receptor types (7), induces apoptosis of pancreatic cancer cells via the same endoplasmic reticulum stress–related proapoptotic pathway as THC (Supplementary Fig. S3). Administration of WIN 55,212-2 not only remarkably reduced the growth of intrapancreatic tumors (Fig. 5A) but also significantly decreased the extension of the tumor cells to proximal (Fig. 5B) and distal (Fig. 5C) organs, indicating that cannabinoid treatment affects the growth and spreading of pancreatic tumor cells.

To investigate the mechanisms involved in this cannabinoid action in vivo, we analyzed the tissue samples obtained in these experiments. Treatment with WIN 55,212-2 increased the number of apoptotic cells in tumor nodules (Fig. 6A) but not in normal pancreatic tissue. To confirm the implication of this gene in the response to THC in these tumors, we first generated pancreatic tumor xenographs by s.c. injection of MiaPaCa2 cells in immuno-deficient mice. As shown in Fig. 4, peritumoral treatment with THC or the CB2-selective (and therefore psychoactivity devoid; ref. 19) cannabinoid agonist JWH-133 reduced notably the growth of the established pancreatic tumors.

Next, we generated tumors by intrapancreatic injection of MiaPaCa2 cells to investigate the antitumoral effect of cannabinoids in a model that resembles more closely the niche of pancreatic cancer spreading. The synthetic cannabinoid agonist WIN 55,212-2 was selected for ip. administration owing to its better bioavailability than THC and other classic cannabinoids. Experiments were done to confirm that this compound, which exhibits affinity for both cannabinoid receptor types (7), induces apoptosis of pancreatic cancer cells via the same endoplasmic reticulum stress–related proapoptotic pathway as THC (Supplementary Fig. S3). Administration of WIN 55,212-2 not only remarkably reduced the growth of intrapancreatic tumors (Fig. 5A) but also significantly decreased the extension of the tumor cells to proximal (Fig. 5B) and distal (Fig. 5C) organs, indicating that cannabinoid treatment affects the growth and spreading of pancreatic tumor cells.

To investigate the mechanisms involved in this cannabinoid action in vivo, we analyzed the tissue samples obtained in these experiments. Treatment with WIN 55,212-2 increased the number of apoptotic cells in tumor nodules (Fig. 6A) but not in normal pancreatic tissue. To confirm the implication of this gene in the response to THC in these tumors, we first generated pancreatic tumor xenographs by s.c. injection of MiaPaCa2 cells in immuno-deficient mice. As shown in Fig. 4, peritumoral treatment with THC or the CB2-selective (and therefore psychoactivity devoid; ref. 19) cannabinoid agonist JWH-133 reduced notably the growth of the established pancreatic tumors.

Next, we generated tumors by intrapancreatic injection of MiaPaCa2 cells to investigate the antitumoral effect of cannabinoids in a model that resembles more closely the niche of pancreatic cancer spreading. The synthetic cannabinoid agonist WIN 55,212-2 was selected for ip. administration owing to its better bioavailability than THC and other classic cannabinoids. Experiments were done to confirm that this compound, which exhibits affinity for both cannabinoid receptor types (7), induces apoptosis of pancreatic cancer cells via the same endoplasmic reticulum stress–related proapoptotic pathway as THC (Supplementary Fig. S3). Administration of WIN 55,212-2 not only remarkably reduced the growth of intrapancreatic tumors (Fig. 5A) but also significantly decreased the extension of the tumor cells to proximal (Fig. 5B) and distal (Fig. 5C) organs, indicating that cannabinoid treatment affects the growth and spreading of pancreatic tumor cells.

To investigate the mechanisms involved in this cannabinoid action in vivo, we analyzed the tissue samples obtained in these experiments. Treatment with WIN 55,212-2 increased the number of apoptotic cells in tumor nodules (Fig. 6A) but not in normal pancreatic tissue.
pancreatic tissue (Fig. 6B) or in the spleen (Supplementary Fig. S4A). In contrast, cannabinoid administration did not significantly modify the percentage of proliferating (proliferating cell nuclear antigen–positive) cells in the tumors (data not shown).

Next, we analyzed the expression of the proapoptotic protein TRB3 because this protein is downstream of p8 and ATF-4 (Fig. 5; refs. 28, 29) and has been proposed to be responsible for the execution of endoplasmic reticulum stress–induced apoptosis (28, 29). WIN 55,212-2 administration increased the expression of TRB3 in tumor nodules (Fig. 6C) but not in normal pancreatic tissue (Supplementary Fig. S4B) or in the spleen (Supplementary Fig. S4C). Taken together, these results indicate that cannabinoid administration selectively induces apoptosis in pancreatic tumor cells in vivo.

Discussion

Despite many years of intensive research, pancreatic cancer remains as the fourth leading cause of cancer death in the United States (1, 2) and the fifth in the Western world overall (3). Current approved therapies based on the administration of fluorouracil chemoradiation for locally advanced tumors and gemcitabine chemotherapy for metastatic disease have only slightly increased the median survival of affected patients (1). In the present report, we show that cannabinoids induce apoptosis of pancreatic tumor cell lines in vitro and exert a remarkable growth-inhibiting effect in models of pancreatic cancer in vivo. The latter effect is evident under various experimental settings (cells inoculated at different sites and cannabinoids administered by different routes). Moreover, our results also show that cannabinoids exert a strong inhibitory effect on the spreading of pancreatic tumor cells not only to adjacent locations such as spleen but also to distal tissues such as liver, diaphragm, stomach, and intestine, thus suggesting that these agents may also decrease the propagation of pancreatic tumor cells.

Although the pancreatic tumor biopsies and cell lines analyzed expressed both CB1 and CB2 cannabinoid receptors, our findings indicate that the CB2 receptor is the one that plays a major role in the proapoptotic effect of cannabinoids in these cells. Previous observations had shown that the CB2 receptor is involved in the antitumoral effect of cannabinoids in gliomas (9, 19), skin carcinomas (11), lymphomas (18), and prostate carcinomas (30), which may be clinically relevant as CB2-selective activation is not linked to the typical marijuana-like psychoactive effects of CB1 activation (7). However, the molecular mechanisms involved in those CB2-mediated actions are only partially understood. Results presented here, together with data obtained in rat glioma and human astrocytoma cells (25), show that p8 and ATF-4 up-regulation mediates cannabinoid-induced apoptosis via induction of the proapoptotic protein TRB3 (29, 31). Of interest, it has been recently shown that ATF-4 regulates TRB3 expression to induce apoptosis of human transformed cells (29). This pathway is triggered by endoplasmic reticulum stress (29), and our results support that it is also involved in cannabinoid-induced apoptosis of
human pancreatic tumor cells in vitro and in vivo. Of potential interest for future cannabinoid-based therapies, cannabinoid treatment does not seem to activate this pathway in normal pancreas or spleen, suggesting that these agents may activate the endoplasmic reticulum stress proapoptotic pathway selectively in tumor cells.

On the other hand, our data also implicate de novo synthesized ceramide in the proapoptotic effect of THC. Many chemotherapeutic agents have been shown to mediate their antiproliferative effects via regulation of ceramide production (32). In line with these observations, we had previously shown that this proapoptotic sphingolipid participates in the antitumoral action of cannabinoids in glioma cells (9, 13, 20). Here we show that de novo synthesized ceramide is involved in THC-induced up-regulation of p8, ATF-4, and TRB3 in pancreatic tumor cells. Interestingly, p8 had been shown to be regulated by ceramide (24, 25) and we have also observed that pharmacologic inhibition of ceramide synthesis de novo decreases basal p8 expression (data not shown). Thus, our findings indicate that the mechanism of cannabinoid-induced apoptosis of human pancreatic tumor cells involves a CB₂ receptor–dependent accumulation of de novo synthesized ceramide that leads to p8, ATF-4, and TRB3 up-regulation (Fig. 6D).

One of the major problems that face cancer therapies is the resistance to chemotherapy. It is worth noting that low doses of THC (but not of WIN 55,212-2) slightly induced proliferation of Panc1 and Capan2, but not of MiaPaca2 and BxPc3 cells, an effect that does not depend on cannabinoid receptors (data not shown). A similar growth-promoting effect of cannabinoids at submicromolar concentrations has previously been described by us (33) and others (34) for several transformed cells. Interestingly, our observations suggest that this behavior may be related with a lower sensitivity to THC proapoptotic action, as higher doses of this compound (although still within the low micromolar range) are necessary to induce CB receptor–dependent apoptosis of the human pancreatic (present study) and astrocytoma⁵ cell lines that exhibit this proliferative response. Thus, the potential development of future cannabinoid-based therapies for this or other types of tumors should ideally evaluate the sensitivity of each particular tumor to these compounds. In our laboratories, we are currently investigating the gene expression profile associated with a higher sensitivity/resistance of tumor cells to cannabinoid treatment to improve the selectivity and efficiency of a potential cannabinoid-based therapy.

In conclusion, results presented here show that cannabinoids exert a remarkable antitumoral effect on pancreatic cancer cells

Figure 6. Cannabinoid treatment increases TRB3 expression and apoptosis in pancreatic tumors but not in normal pancreatic tissue. Intrapancreatic tumors were generated as described in Materials and Methods. Animals were treated with either vehicle or WIN 55,212-2 (1.5 mg/kg for 2 days, 2.25 mg/kg for 2 additional days, and 3.0 mg/kg for 10 additional days; n = 6 for each experimental group). The day after the 14-day treatment, animals were sacrificed, pancreatic tissues containing tumors were fixed, and sections were prepared. A, representative images (×40) of TUNEL-stained pancreatic tumors. Values in the lower left corner of the bottom images correspond to 12 sections of three dissected tumors for each condition and are expressed as the percentage of TUNEL-positive cells relative to the total number of cells in each section. B, representative images (×20) of TUNEL-stained pancreas. T, tumor tissue; P, normal pancreatic tissue. C, representative images (×40) of TRB3-stained pancreatic tumors. Values in the lower left corner of the bottom images correspond to 18 sections of three dissected tumors for each condition and are expressed as the percentage of TRB3-stained area relative to the total area in each section. D, schematic representation of the proposed mechanism of cannabinoid-induced apoptosis on pancreatic tumor cells.

Authors’ unpublished observations.
in vitro and in vivo due to their ability to selectively induce apoptosis of these cells via activation of the p8-ATF4-TRB3 proapoptotic pathway. These findings may help to set the basis for a new therapeutic approach for the treatment of this deadly disease.

Acknowledgments

Received 1/16/2006; revised 4/5/2006; accepted 5/5/2006.

References

Cannabinoids Induce Apoptosis of Pancreatic Tumor Cells via Endoplasmic Reticulum Stress–Related Genes

Arkaitz Carracedo, Meritxell Gironella, Mar Lorente, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/13/6748

Cited articles
This article cites 34 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/13/6748.full.html#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/66/13/6748.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.