The Cannabinoid WIN 55,212-2 Decreases Specificity Protein Transcription Factors and the Oncogenic Cap Protein eIF4E in Colon Cancer Cells

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Abstract

2,3-Dihydro-5-methyl-3-([morpholinyl][methyl]pyrollo(1,2,3-de)-1,4-benzoxazinyl]-[1-naphthalenyl]methanone [WIN 55,212-2, (WIN)] is a synthetic cannabinoid that inhibits RKO, HT-29, and SW480 cell growth, induced apoptosis, and downregulated expression of survivin, cyclin D1, EGF receptor (EGFR), VEGF, and its receptor (VEGFR1). WIN also decreased expression of specificity protein (Sp) transcription factors Sp1, Sp3, and Sp4, and this is consistent with the observed downregulation of the aforementioned Sp-regulated genes. In addition, we also observed by RNA interference (RNAi) that the oncogenic cap protein eIF4E was an Sp-regulated gene also downregulated by WIN in colon cancer cells. WIN-mediated repression of Sp proteins was not affected by cannabinoid receptor antagonists or by knockdown of the receptor but was attenuated by the phosphatase inhibitor sodium orthovanadate or by knockdown of protein phosphatase 2A (PP2A). WIN-mediated repression of Sp1, Sp3, and Sp4 was due to PP2A-dependent downregulation of microRNA-27a (miR-27a) and induction of miR-27a–regulated ZBTB10, which has previously been characterized as an “Sp repressor.” The results show that the anticancer activity of WIN is due, in part, to PP2A-dependent disruption of miR-27a:ZBTB10 and ZBTB10-mediated repression of Sp transcription factors and Sp-regulated genes, including eIF4E. Mol Cancer Ther; 12(11); 2483–93. ©2013 AACR.

Introduction

There are three major classes of cannabinoids, which include plant-derived compounds such as Δ(9)-tetrahydrocannabinol (THC) and cannabidiol, endogenous cannabinoids (anandamide and 2-arachidonylglycerol), and synthetic compounds that mimic the effects of cannabinoids (1, 2). Cannabinoids bind the CB1 and CB2 receptors, which are differentially expressed in some but not all tissues, and there is also evidence that some cannabinoids also interact with other G-protein–coupled receptors (GPCR) including the receptor transient receptor potential vanilloid type I (TRPV1) and GPR55 (3–5). THC is the main psychoactive cannabinoid found in the marijuana plant Cannabis sativa L; however, in addition to the psychoactive effects of THC, endocannabinoids and synthetic cannabinoids also play a role in energy metabolism, pain and inflammation, cardiovascular, musculoskeletal and respiratory disorders, and cancer, and opportunities for cannabinoid-based pharmacotherapies are extensive (2).

The anticancer activities of cannabinoids have been known for over 3 decades, and clinical trials for treatment of gliomas with cannabinoids have been reported (6). Cannabinoids inhibit growth, induce apoptosis, and exhibit antmitotic and antiangiogenic activities in multiple cancer cell lines and inhibit tumor growth in in vivo mouse models (7–10). Cannabinoids are active in the tumor microenvironment and the effects of cannabinoids are complex and dependent on ligand structure and cell context, and the responses can also be cannabinoid (CB; CB1 and/or CB2) receptor–dependent or -independent (7–10). For example, the synthetic mixed CB1 and CB2 receptor agonist 2,3-dihydro-5-methyl-3-([mor-pholinyl][methyl]pyrollo(1,2,3-de)-1,4-benzoxazinyl]-[1-naphthalenyl]methanone [WIN 55,212-2, (WIN)] inhibited gastric cancer cell growth and decreased VEGF expression and these responses were blocked in cells cotreated with WIN plus CB receptor antagonists (11), and similar CB receptor–dependent responses were observed in mantle cell lymphoma (12). In contrast, WIN induced phosphatase-dependent apoptosis in SW480 colon and LNCaP prostate cancer cells and these responses were inhibited by the phosphatase inhibitor sodium orthovanadate (SOV) but not by CB receptor antagonists or by CB receptor knockdown (13).
Materials and Methods

Chemicals, antibodies, and reagents

WIN 55,212-2 mesylate (1038), AM251 (1117), and AM630 (1120) were purchased from Tocris Bioscience. Tyrosine phosphatase inhibitor, SOV, was purchased from Calbiochem. Dithiothreitol (DTT) was obtained from Sigma-Aldrich. Antibodies for EGFR, Sp3, and Sp4, which are overexpressed in colon and other cancer cell lines and tumors; moreover, knockdown of Sp proteins by RNAi results in growth inhibition and induction of apoptosis (27–39). Therefore, we hypothesized that the anticancer activity of cannabinoids such as WIN in colon cancer cells may also be due, in part, to downregulation of Sp proteins and pro- oncogenic Sp-regulated genes and this could also be related to the reported activation of phosphatases by cannabinoids in these cells (13). Results of this study show that WIN induced protein phosphatase 2A (PP2A)-dependent downregulation of Sp1-, Sp3-, Sp4-, and Sp-regulated gene products including the pro-oncogenic cap protein eIF4e in colon cancer cells. These responses were CB receptor-independent and involved disruption of microRNA-27a (miR-27a)–mediated suppression of the zinc finger transcriptional repressor ZBTB10, which acts as an Sp repressor (31, 33–35).

Methods

Cell proliferation assay

Colon cancer cells (1 × 10^5 per well) were seeded in 12-well plates and allowed to attach for 24 hours. The medium was then changed to DMEM/Ham F-12 medium containing 2.5% charcoal-stripped FBS and either vehicle (dimethyl sulfoxide, DMSO) or varying concentrations of WIN was added. Cells were stained after 9 hours with fluorescein isothiocyanate (FITC)–Annexin V, propidium iodide, and 4′,6-diamidino-2-phenylindole (DAPI) dyes according to the manufacturer’s protocol and were visualized under EVOS fl fluorescence microscope, from Advanced Microscopy Group using a multiband filter set for FITC, rhodamine, and DAPI. The proportion of apoptotic cells was determined by the amount of green fluorescence observed.

Terminal deoxyribonucleotide transferase-mediated nick-end labeling assay

HT-29 (7 × 10^4) were seeded in four-chambered glass slides and left overnight to attach. After treatment with WIN for 48 hours, the in situ cell death detection POD kit was used for the terminal deoxyribonucleotide transferase-mediated nick-end labeling (TUNEL) assay according to the manufacturer’s protocol. The percentage of apoptotic cells was determined by counting stained cells from eight fields, each containing 50 cells.

Western blot analyses

Colon cancer cells were seeded in DMEM/Ham F-12 medium and were allowed to attach for 24 hours. Cells were treated with either DMSO or WIN for indicated time periods or pretreated with the proteasome inhibitors, antioxidants, phosphatase inhibitors, and CB receptor inhibitors.

References

For a complete list of references, please see the original article.
antagonists, and then treated with WIN. Cells were lysed and analyzed by Western blot analysis as described previously (13).

**siRNA interference assay**
Colon cancer cells were seeded (2 × 10^5 per well) in six-well plates in DMEM/Ham F-12 medium supplemented with 2.5% charcoal-stripped FBS without antibiotics and left to attach for 24 hours. siRNAs specific for the CB1 and CB2 receptors, PP2A, and ZBTB10 along with iLamin/iGL2 as control were transfected using Lipofectamine 2000 reagent according to the manufacturer’s protocol.

**Quantitative real-time PCR**

SW480 colon cancer cells were plated (2 × 10^5) and left to attach for 24 hours. Cells were transfected with siRNAs for PP2A and then treated with either DMSO or WIN for 24 and 48 hours. miRNA was isolated using the mirVana miRNA isolation kit from Ambion-Life Technologies according to the manufacturer’s protocol. cDNA was prepared using the Taqman MicroRNA Reverse Transcription Kit and was subjected to quantitative real-time PCR (qRT-PCR) with specific primers for miR-27a using the Taqman Universal PCR Master Mix from Applied Biosystems in the CFX384 Real-Time PCR Detection System from Bio-Rad.

**PP2A phosphatase assay**

Cells were seeded (3 × 10^5) and left to attach for 24 hours. Cells were treated with DMSO, okadaic acid, and 5 and 7.5 μmol/L WIN. Cells were harvested and lysed using the high salt lysis buffer. The lysates were then subjected to buffer exchange using the Zeba Desalt Spin Columns (8981) from Thermo Scientific to eliminate any contaminating phosphates that could skew the experimental results. The lysates were then immunoprecipitated with anti-PP2A, C subunit, antibody, and were incubated in a mixture of diluted phosphopeptidase and serine/threonine assay buffer for 10 minutes at 30°C in a shaking incubator. The phosphatase activity was determined by addition of malachite green dye and comparing the absorbance between controls and treatment at 650 nm in a plate reader.

**Results**

Treatment of SW480, RKO, and HT-29 colon cancer cells with 2.5, 5.0, and 7.5 μmol/L WIN for 24 or 48 hours significantly inhibited cell proliferation at the two higher concentrations (Fig. 1A–C), whereas minimal growth inhibition was observed for nontransformed CCD-18Co colon cells (Supplementary Fig. S1A). In addition, WIN also induced Annexin V staining in SW480 and RKO cells within 9 hours after treatment, indicating rapid induction of early apoptosis, whereas Annexin V was not induced in HT-29 cells at the early time-point (data not shown). In contrast, TUNEL staining was increased in HT-29 cells treated with WIN for 48 hours, indicating differences between the timing of WIN-induced apoptosis in SW480/RKO versus HT-29 cells. The red staining in RKO cells treated with 7.5 μmol/L WIN was associated with dead cells and necrosis (Fig. 1B). Figure 1D shows that treatment with WIN for 48 hours also induced PARP cleavage in all three colon cancer cell lines as previously reported for WIN in SW480 cells (13).

WIN-induced growth inhibition and apoptosis was also accompanied by decreased expression of growth-promoting (EGFR, cyclin D1) and survival (survivin) genes’ products in SW480, RKO, and HT29 cells (Fig. 2A). Treatment of SW480, RKO, and HT29 cells with 2.5, 5.0, or 7.5 μmol/L WIN decreased levels of VEGF and VEGFR1 protein in the three cell lines, although the effects in HT-29 cells were less than those observed in the other two cell lines (Fig. 2B). Treatment of SW480, RKO, and HT-29 cells with 2.5, 5.0, or 7.5 μmol/L WIN for 48 hours decreased expression of Sp1, Sp3, and Sp4 proteins, as indicated by quantitation of the band intensities (relative to β-actin; Fig. 2C). Both the high and low molecular weight forms of Sp3 were observed, as previously reported in colon cancer cell lines (37–39). This is the first example of a synthetic cannabinoid decreasing expression of Sp proteins and Sp-regulated gene products in cancer cells; however, we have recently reported that betulinic acid, a triterpenoid compound that also decreases Sp1, Sp3, and Sp4 protein levels in multiple cancer cell lines, exhibits CB receptor agonist activity (35).

Drug-induced downregulation of Sp transcription factors has been linked to activation of proteasomes or induction of reactive oxygen species (ROS; refs. 31, 36–39). Using SW480 cells as a model, we show that WIN-induced downregulation of Sp1, Sp3, and Sp4 was not reversed in cells cotreated with WIN plus the proteasome inhibitors lactacystin or gliotoxin (Fig. 3A). Moreover, treatment of SW480 cells with WIN in combination with the antioxidants DTT or GSH also did not inhibit WIN-induced downregulation of Sp1, Sp3, or Sp4 proteins (Fig. 3B), whereas betulinic acid and GT-094 (a nitro-NSAID)-induced downregulation of Sp1, Sp3, and Sp4 in SW480 cells was significantly inhibited after cotreatment with the antioxidants (38, 39). The CB1 and CB2 receptor antagonists AM251 and AM560 had minimal effects on Sp protein expression and, in combination with WIN, did not inhibit WIN-induced downregulation of Sp1, Sp3, or Sp4 proteins (Fig. 3C). Moreover, similar results were observed in SW480 cells after knockdown of the CB1 (iCB1) or the CB2 (iCB2) receptor by RNAi (Fig. 3D), showing that the effects of WIN on downregulation of Sp transcription factors was CB receptor-independent.

WIN induced several phosphatase mRNAs in SW480 cells and the phosphatase inhibitor SOV partially blocked WIN-induced PARP cleavage (13) and, therefore, we investigated the effects of SOV on WIN-induced downregulation of Sp1, Sp3, and Sp4 proteins using SW480 cells as a model. Figure 4A shows that WIN-induced downregulation of Sp1, Sp3, and Sp4 was reversed after cotreatment with SOV and these results are consistent with the inhibition of WIN-induced PARP cleavage by SOV (13) as
knockdown of Sp1 (by RNAi) also enhances PARP cleavage (31). A recent study showed that 
\( \alpha \)-tocopherol succinate–induced downregulation of Sp1 in prostate cancer cells was PP2A-dependent (40) and Western blot analysis of whole-cell lysates from SW480 cells transfected with a nonspecific oligonucleotide (iCtrl) or a specific oligonucleotide that targets PP2A (iPP2A) and treated with 7.5 \( \mu \)mol/L WIN or DMSO showed that WIN-mediated downregulation of Sp1, Sp3, and Sp4 proteins was significantly attenuated in cells transfected with iPP2A (Fig. 4B). Similar results were also observed in RKO cells with some differences in the relative effectiveness of PP2A knockdown (Supplementary Fig. S1B). PP2A knockdown also attenuated WIN-induced downregulation of cyclin D1 and VEGFR1 but had minimal effect on EGFR protein levels (Fig. 4C), suggesting that decreased expression of EGFR in SW480 cells treated with WIN was phosphatase- and Sp-independent.

We also used an in vitro assay to confirm that WIN induced PP2A activity because previous studies did not observe induction of PP2A mRNA levels in SW480 cells treated with WIN (13). PP2A activity in SW480 was measured using the PP2A Immunoprecipitation Phosphatase Assay Kit from Millipore. Treatment of SW480 cells with 7.5 \( \mu \)mol/L WIN induced an approximate 30% increase in PP2A activity and this was comparable with the induction of PP2A phosphatase activity by metformin (41). Figure 4D shows that 10 nmol/L okadaic acid...
significantly inhibited PP2A activity in SW480 cells (positive control). Cell lysates used for the phosphatase activity assays were then subjected to Western blot analysis, and expression of Sp1, Sp3, and Sp4 protein was quantitated (C) relative to β-actin (levels in the DMSO group were set at 1.0). Results are expressed as the mean ± SE (three replicate experiments), and significantly (P < 0.05) decreased proteins are indicated (*). Data in Figures 2A and 2B are from the same experiment.

Figure 2. WIN downregulates Sp-regulated proliferative, survival (A), and angiogenic (B) gene products and Sp proteins (C) in SW480, RKO, and HT-29 cells. Cells were treated with 2.5 to 7.5 μmol/L of WIN for 48 hours, whole-cell lysates were analyzed by Western blot analysis, and expression of Sp1, Sp3, and Sp4 protein was quantitated (C) relative to β-actin (levels in the DMSO group were set at 1.0). Results are expressed as the mean ± SE (three replicate experiments), and significantly (P < 0.05) decreased proteins are indicated (*). Data in Figures 2A and 2B are from the same experiment.

PP2A decreases expression of the phosphorylated form of the pro-oncogenic cap protein eIF4E (p-eIF4E; ref. 42) and therefore the time-dependent effects of 7.5 μmol/L WIN on expression of Sp1, Sp3, Sp4, p-eIF4E, and eIF4E proteins were investigated (Fig. 5A). Sp1, Sp4, and Sp3 (high molecular weight forms) expression was significantly decreased after 12 hours, whereas the lower molecular weight forms of Sp3 were more slowly decreased over the 48-hour period. P-eIF4E and eIF4E proteins significantly decreased within 12 hours after treatment with WIN and continued to decrease (over 48 hours); the decrease of eIF4E (total protein) was more gradual than observed for the phospho-protein. Treatment of SW480 cells with WIN alone or in combination with SOV showed that the phosphatase inhibitor blocked WIN-induced effects on p-eIF4E and eIF4E, but not PP2A (Fig. 5B), showing that WIN-induced downregulation of p-eIF4E, eIF4E, Sp1, Sp3, and Sp4 was phosphatase dependent. The role of PP2A in this process was investigated by RNAi, and WIN-mediated repression of p-eIF4E and eIF4E (total) proteins was significantly inhibited by knockdown of PP2A (Fig. 5C), showing a role for PP2A in mediating downregulation of eIF4E and pE4E and this parallels results observed for phosphatase-dependent downregulation of Sp transcription factors (Fig. 4), suggesting possible cross-regulation of eIF4E and Sp transcription factors. WIN also decreased PP2A protein levels after treatment for 48 hours compared with 24 hours (Fig. 4D) and this is currently being investigated. Knockdown of Sp1, Sp3 (iSp3), and Sp4 (iSp4) in SW480 cells showed that iSp1 and, to a lesser extent, iSp3 also decreased eIF4E protein expression.
(Fig. 5D), whereas Sp4 knockdown had minimal effects, suggesting that in SW480 cells, eIF4E expression is primarily regulated by Sp1. The knockdown of the Sp transcription factors was specific for Sp4 and Sp3; however, iSp1 also decreased expression of Sp4 as previously reported, indicating that Sp4 is an Sp1-regulated gene in SW480 cells (43). In contrast, knockdown of eIF4E by RNAi decreased expression of the targeted protein and slightly increased expression of Sp1, Sp3, or Sp4 proteins, suggesting that cross-regulation between Sp transcription factors and eIF4E is unidirectional. A comparable experiment was carried out in RKO cells; iSp1, iSp3, and iSp4 decreased eIF4E protein expression and the combination of all 3 oligonucleotides caused a marked decrease of eIF4E protein (Supplementary Fig. S1C), indicating that eIF4E is an Sp-regulated gene in colon cancer cells.

Drug-induced proteasome-independent downregulation of Sp1, Sp3, and Sp4 has been related to transcriptional repression because of downregulation of miR-27a and miR-20a/miR-17-5p, which regulate the “Sp repressors” ZBTB10 and ZBTB4 (34, 44). Treatment of SW480 cell with 7.5 μmol/L WIN induced ZBTB10 protein within 12 hours and induction was high after 24 hours but markedly decreased after 36 and 48 hours (Fig. 6A). In contrast, induction of ZBTB4 protein was not observed (data not shown). SW480 cells were treated with 7.5 μmol/L WIN and also transfected with siRNAs against CB1 and CB2 receptors, followed by treatment with WIN for 48 hours, and whole-cell lysates were analyzed by Western blot analysis. D, cells were transfected with siRNAs against CB1 and CB2 receptors, followed by treatment with WIN for 48 hours, and whole-cell lysates were analyzed by Western blot analysis. iLamin was used as a control oligonucleotide.
Supplementary Fig. S1D and S1E). These results show that WIN-mediated downregulation of Sp proteins in SW480 cells is due to activation of PP2A and PP2A-dependent disruption of the miR-27a:ZBTB10, which results in induction of ZBTB10 and ZBTB10-dependent repression of Sp proteins and Sp-regulated genes.

**Discussion**

Sp1, Sp3, and Sp4 proteins are highly expressed in multiple cancer cell lines and tumors, whereas levels of these transcription factors are low to nondetectable in...
nontumor tissue (29–33), and these observations are consistent with reports that Sp1 expression in rodent and human tissues decreases with age (46, 47). The pro-oncogenic activity of Sp proteins is primarily due to Sp-regulated genes, which include several genes that play pivotal roles in cancer cell proliferation (cyclin D1, c-Met, EGFR), survival (survivin, bcl-2), angiogenesis [VEGF and its receptors (VEGFR1 and VEGFR2)], and inflammation (NF-κB, p65). Thus, Sp transcription factors are an excellent example of nononcogene addiction by cancer cells (48) and therefore an important target for mechanism-based anticancer drugs. Reports from different laboratories have identified diverse agents that decrease expression of Sp transcription factors and these include various phytochemical anticancer compounds including betulinic acid and their synthetic analogs, NSAIDs, bortezimob,
α-tocopherol succinate, arsenic trioxide, and other ROS inducers (29–40, 49).

Betulinic acid decreases expression of Sp1, Sp3, and Sp4 in prostate, colon, bladder, and breast cancer cell lines (32, 35, 37, 38). Results illustrated in Figs. 1 and 2 confirm that the cannabinoid WIN also inhibited growth and induced apoptosis in SW480, RKO, and HT-29 colon cancer cells and downregulated Sp1-, Sp3-, Sp4-, and Sp-regulated genes. Because knockdown of Sp1 or multiple Sp transcription factors alone in cancer cells inhibits growth and induces apoptosis (29–32), the anticancer activity of WIN is due, in part, to downregulation of Sp proteins. However, in contrast with betulinic acid and other agents that downregulate Sp transcription factors, WIN-induced Sp downregulation was proteasome- and ROS-independent and knockdown or inhibition of CB1 or CB2 receptors did not affect this response in SW480 cells (Fig. 3). The receptor-independent effects of WIN were consistent with previous studies showing that the anticancer activity of WIN was both receptor-dependent and -independent (11–13).

We recently reported that WIN-induced apoptosis in SW480 and LNCaP cells is inhibited by the phosphatase inhibitor SOV, but not by CB receptor knockdown, and WIN also induces multiple phosphatase mRNAs (13). These results, coupled with a report that PP2A plays a role in α-tocopherol succinate–induced downregulation of Sp1 in prostate cancer cells (40), prompted us to investigate the effects of SOV and PP2A knockdown on WIN-induced repression of Sp proteins. Both SOV and PP2A
knockdown attenuated WIN-mediated downregulation of Sp1, Sp3, Sp4, and Sp5-regulated gene products (Fig. 4), confirming a role for PP2A and possibly other unidentified phosphatases in mediating the anticancer activities of WIN. An important proteasome-independent pathway for drug-induced downregulation of Sp1, Sp3, and Sp4 involves induction of the transcriptional repressors ZBTB10 and ZBTB4, which exhibit low expression in cancer cell lines due to their regulation (repression) by miR-27a and miR-20a/miR-17-5p, respectively (38, 39, 44, 49). WIN did not induce ZBTB4 in SW480 cells; however, a time-dependent induction of ZBTB10 protein was observed (Fig. 6A) and this response was abrogated after knockdown of PP2A (Fig. 6B), and these results were paralleled by PP2A-dependent repression of miR-27a (Fig. 6C). Moreover, because knockdown of ZBTB10 by RNAi blocks WIN-mediated downregulation of Sp1, Sp3, and Sp4 proteins (Fig. 6D) and because miR-27a antagonists and ZBTB10 expression decrease Sp protein expression in colon cancer cells (49), it is clear that disruption of miR-27a/ZBTB10 is critical for the observed responses, and results of c-Myc and p65(NF-kB) silencing did not decrease miR-27a expression (Supplementary Fig. S2). The mechanism of PP2A-mediated effects on miR-27a/ZBTB10 is unknown and are currently being investigated.

The induction of PP2A activity by WIN suggested that WIN may also regulate phosphorylation of the important cap protein eIF4E as it has been reported that PP2A dephosphorylates p-eIF4E, which has been characterized as a pro-oncogenic phosphoprotein (42). WIN clearly decreased expression of p-eIF4E; however, this was unexpectedly accompanied by downregulation of eIF4E (total protein). Because eIF4E and Sp transcription factors regulate expression of several genes in common (e.g., cyclin D1 and VEGF), we used RNAi to show for the first time that eIF4E is an Sp-regulated gene in colon cancer cells (Fig. 5D). This observation extends the number of pro-oncogenic factors that are regulated by Sp1, Sp3, and Sp4 in cancer cells, emphasizing the potential clinical applications of drugs that target Sp proteins. Previous studies show that other cannabinoids such as cannabidiol, other synthetic cannabinoids, and endocannabinoids inhibit colon cancer cell and tumor growth (22–26), and in vivo studies show that loss of the CB1 receptor enhances intestinal adenoma growth in APCmin/+ mice (23). The effects of these compounds on Sp transcription factors and eIF4E expression and the role of the CB receptors in mediating these responses are currently being investigated.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: S. Sreevalsan, S. Safe
Development of methodology: S. Sreevalsan, S. Safe
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Sreevalsan, S. Safe
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Writing, review, and/or revision of the manuscript (S. Sreevalsan, S. Safe)
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References
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