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EXPERIMENTAL STUDY

Anti-cancer effects of selective cannabinoid agonists in pancreatic and breast cancer cells

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ABSTRACT

OBJECTIVE: Cancer ranks first among the causes of morbidity and mortality all over the world, and it is expected to continue to be the main cause of death in the coming years. Therefore, new molecular targets and therapeutic strategies are urgently needed. In many cases, some reports show increased levels of endocannabinoids and their receptors in cancer, a condition often associated with tumour aggressiveness. Recent studies have suggested that cannabinoid-1/2 receptors contribute to tumour growth in a variety of cancers, including pancreatic, colon, prostate, and breast cancer. Understanding how cannabinoids can regulate key cellular processes involved in tumorigenesis, such as: cell proliferation and cell death, is crucial to improving existing and new therapeutic approaches for the cancer patients. The present study was aimed to characterize the in-vitro effect of L-759633 (a selective CB2 receptor agonist), ACPA (a selective CB1 receptor agonist) and ACEA (a selective CB1 receptor agonist) on the cell proliferation, clonogenicity, and apoptosis in pancreatic (PANC1) and breast (MDA-MB-231) cancer cells.

METHODS: The viability and/or proliferation of cells were detected by MTS assay. A clonogenic survival assay was used to detect the ability of a single cell to grow into a colony. Apoptosis was determined with Annexin V staining (Annexin V-FITC/PI test) and by analyzing the expression of Bcl-2-associated X protein (Bax) and B-cell lymphoma 2 (Bcl-2).

RESULTS: We found that selective CB1/2 agonists suppressed cell proliferation, clonogenicity and induced proapoptotic function in human PANC1 pancreatic and MDA-MB-231 breast cancer cells. Based on our findings, these agonists led to the inhibition of both cell viability and clonogenic growth in a dose dependent manner. CB1/2 agonists were observed to induce intrinsic apoptotic pathway by upregulating Bax, while downregulating Bcl-2 expression levels.

CONCLUSION: Our data suggests that CB1/2 agonists have the therapeutic potential through the inhibition of survival of human PANC1 pancreatic and MDA-MB-231 breast cancer cells and also might be linked with further cellular mechanisms for the prevention *(Fig. 5, Ref. 49)*. Text in PDF *www.elis.sk*

KEY WORDS**:** L-759633, ACPA, ACEA, apoptosis, pancreatic cancer, breast cancer.

Introduction

Cancer is the second leading cause of death after cardiovascular diseases in many countries (1). Over the past years, there has been an increasing trend in cancer cases and cancer-related deaths, particularly in low- and middle-income countries, depending on the diversity of lifestyle, diverse habits, and geographic and environmental factors (2). Especially in recent years, there have been great developments in treatment strategies targeting various biomolecules in cancer cells for the treatment of cancer.

Cannabinoids are the group of chemicals known as marijuana and obtained from the plant called "*Cannabis sativa linnaeus*". The major active ingredient in *Cannabis sativa linnaeus* is delta-9-tetrahydrocannabinol (Δ^9-THC) with lipophilic properties which acts by mimicking the effects of endogenous cannabinoids (3). Cannabinoids have been used for therapeutic purposes with the discovery of their mechanism of action, agonists and antagonists of their receptors (4–6). First, in 1975, Munson et al. showed that Δ^9-THC improved survival in the lung cancer xenograft model (7). After this study, numerous studies have been carried out on many biological effects of cannabinoids for cancer **(**8–12).

The effects of cannabinoids on signalling pathways in cancer cells are mediated via G-protein coupled cannabinoid receptors (CB receptors), CB1 receptor and CB2 receptor, as well as through other receptors and in a receptor-independent manner. Both receptors are highly expressed in different cancer tissues. CB1/2 receptors belong to the seven transmembrane spanning receptor superfamily. CB1 receptors are abundant in the brain areas related to anxiety, memory, motor coordination, pain, and endocrine functions. CB1 receptors are also expressed in other sites such as

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uterus, testis, and spleen (13). CB1 receptor activation inhibits forskolin stimulated adenylyl cyclase through stimulating cellular signal transduction via Gi/o, to inhibit calcium channels (P, Q, N type). CB2 receptors are expressed in the immune system organs and cells. CB2 receptors are also found in the pancreas, bones, heart, liver, and endothelium, at a lower level. CB2 receptor activation strongly stimulates Gi, leading to adenylate cyclase inhibition (14). The survival and growth of tumour cells often depends on increased signalling through pathways that regulate cell proliferation and survival. Activation of cannabinoid receptors additionally leads to activation of phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (15, 16). As both CB1/2 receptors are highly expressed in different cancer tissues, these receptors are emerging targets for cancer treatment, even though their exact role in cancer progression is still not completely known. The general consensus in literature suggests that cannabinoids have anticancer effects. It has been indicated that different cannabinoids inhibit cell proliferation in vitro and tumour growth in vivo and that in the mechanism of these effects, apoptosis has a significant role (17-20).

Cannabinoid therapy promotes cancerous cell death, reduction of tumour angiogenesis, as well as blockade of invasion and metastasis. Cannabinoid anticancer mechanism is substantially related to inducing autophagy mediated apoptotic cancer cell death (21, 22). In addition to the cancer cell death, treatment with cannabinoids has been shown to normalize the tumour vasculature, which is considered to be based on the inhibition of the endothelial growth factor (VEGF) pathway. Again, cannabinoids have been shown to reduce distant tumour mass formation in animal models with metastasis. These compounds have been shown to inhibit migration, and invasion in different types of cancer cells (23–25). Regulation of extracellular proteases and their inhibitors also contribute to the antimetastatic effects of cannabinoids (24, 26). These items also show an acceptable safety profile. However, there are still conflicting results regarding the anticancer effects of cannabinoids and new studies are needed on the mechanism of this effect. In this study, we aimed to evaluate the in-vitro impact of L-759633 (a selective CB2 receptor agonist), ACPA (a selective CB1 receptor agonist) and ACEA (a selective CB1 receptor agonist) on cell proliferation, clonogenity and apoptosis against pancreatic (PANC1) and breast (MDA-MB-231) cancer cells.

Material and methods

Cell culture and reagents

The human breast and pancreas cancer cell lines (MDA-MB-231 and PANC1) employed were obtained from American Type Culture Collection (ATCC, Manassas, VA). All the cell lines were cultured in DMEM/F12 (Life Technologies, Gibco BRL, Grand Island, NY) supplemented with 10 % FBS (FBS, Hyclone). Media was supplemented with penicillin and streptomycin (100 U/mL, Invitrogen). The cells were cultured under standard conditions at 37 °C in a humidified incubator containing 5 % CO_2 and were used between passages 4 and 15. CB2-agonist-L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA were purchased from Tocris Bioscience (Wiesbaden-Nordenstadt, Germany).

Cell viability and proliferation assays

Cell viability and proliferation was evaluated by MTS assay (Promega, Madison, WI, USA). A hemocytometer was used for cell count and viable cells were identified by trypan blue exclusion. Cells ($1.5x10^3$ cells/well) were seeded in 96-well plates and treated with CB2-agonist -L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA at a dose range between 1-250 μM for 72 h. Following treatment, a solution which contained MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) and PMS (phenazine methosulfate) (20:1 v/v) was applied to each cell at 37 °C during 3 h. For viable growing cell estimation, the absorbance was read at 490 nm. Experiments were carried out in triplicate and the results were presented as the mean absorption ± standard deviation.

Clonogenic survival assay

This assay is a cell survival and proliferation assay, which is based on a single cell growing into a colony in vitro (27). Gently mixed 500 cells were plated on a 6 well culture plate. Following incubation for 24 hours, cells were treated with 50 and 100 μM doses of CB2-agonist -L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA once a week and grown for 2–3 weeks. Then cells were washed with phosphate-buffered saline and crystal violet staining was performed. Colonies greater than 50 cells in diameter were counted. Experiments were done in triplicate.

Western blot analysis

For analysis of Bcl-2-associated X protein (Bax) and B-cell lymphoma 2 (Bcl-2) levels, cells were seeded in $5x10^5$ cells/culture flasks. After 72 h treatment with control and test compounds, cells were collected and centrifuged, then washed twice in ice cold phosphate buffered saline (PBS). Cells were lysed in a lysis buffer at 4 °C, lysates were centrifuged at 13,000x g for 10 min and collected supernatant fractions were used for Werstern blot analysis. For each sample, a total protein concentration was determined by detergent protein assay kit (Bio-Rad, Hercules, CA). Quantification of proteins was performed on 40 μg protein/lane on 4–15 % SDS-PAGE gels. After electrophoretic transfer to PVDF membranes, the membranes were blocked for 60 min in a blocking buffer (0.1 % Triton X-100 with 5 % non-fat dry milk in TBS-Tween 20). Following wash with TBS-T (diluted in TBS-Tween 20 containing 5 % non-fat dry milk, and incubated at 4 °C overnight), membranes were probed with primary antibodies Bax and Bcl-2(Cell Signalling Technology, Danvers, MA). Wash with TBS-T was followed by incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit and anti-mouse secondary antibody (Cell Signalling Technology, Danvers, MA). As a loading control, the mouse anti-β-actin antibody (Sigma Chemical, St. Louis, MO) was used to observe β-actin expression. For chemiluminescent detection, chemi-glow detection reagents were used (Alpha Innotech, San Leandro, CA). FluorChem 8900 imager was used for visualizing blots and blots were quantified by densitometric

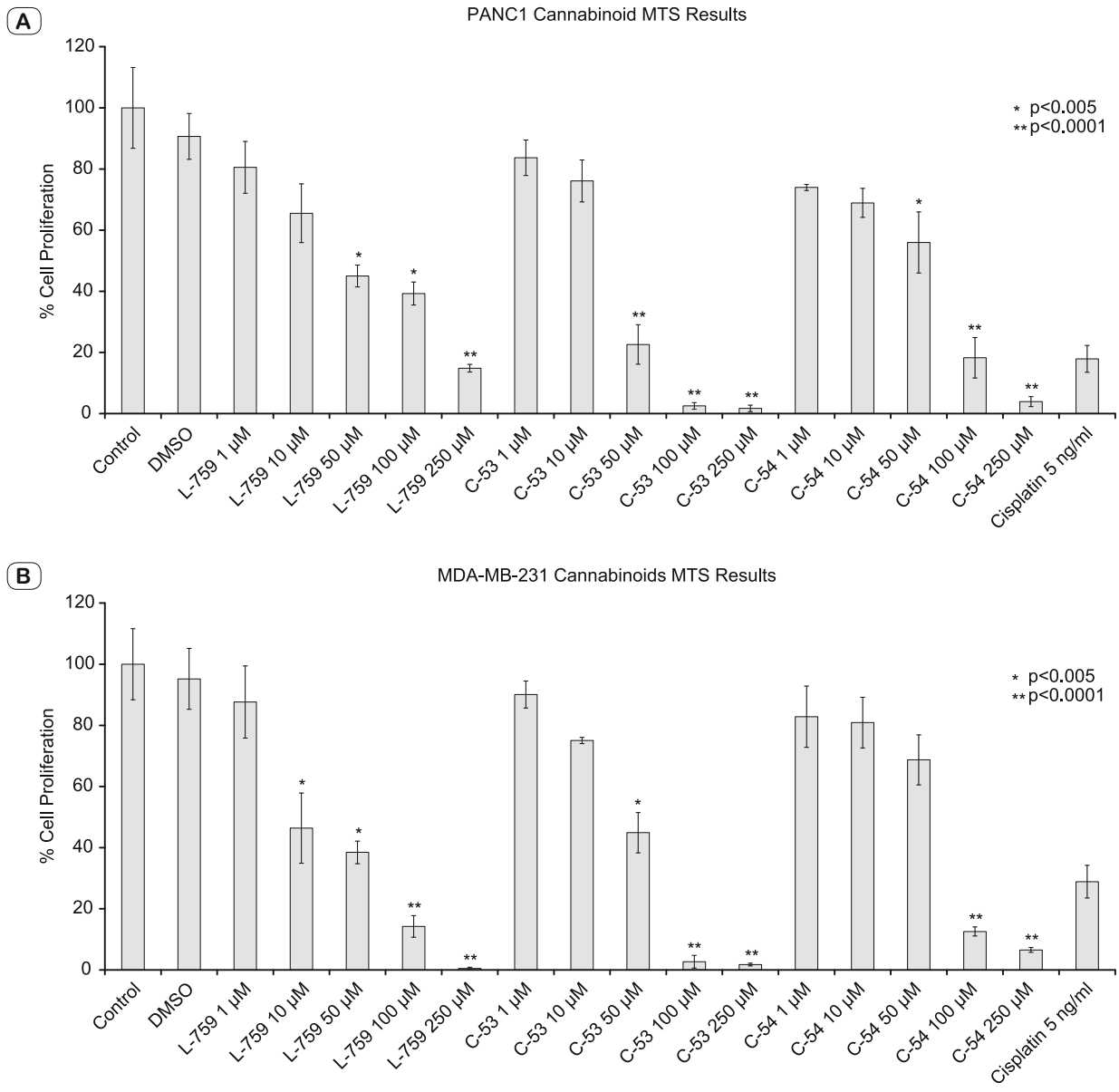

Fig. 1. Effect of increasing concentrations of selective CB2-agonist (L-759633), CB1-agonist-ACPA and CB1-agonist-ACEA agonists on cell proliferation (A) in human PANC1 pancreatic cancer cells and (B) in human MDA-MB-231 breast cancer cells measured by MTS assay. Cells were treated with cannabinoid agonists at a dose range between 1-250 µM or cisplatin 5 ng/ml for 72 h. Data are expressed as the mean (± SD) values. * p < 0.005; ** p < 0.0001 compared to the control group.

scanning using an image analysis program (ImageJ 1.48s processing software, National Institutes of Health, Bethesda, MD, USA). Experiments were performed at least twice.

Analysis of cell death

Apoptosis was evaluated by an Annexin V assay. Cells were seeded in 25-cm^2 culture flasks (5x10^5 cells/flask). The cells were treated with indicated CB2-agonist -L-759633 at doses of 10 and 50 µM, CB1-agonist-ACPA and CB1-agonist-ACEA at dose of 50 µM for 72 h, and then analyzed by Annexin V to identify apoptotic cells and propidium iodide (PI) staining to distinguish viable cells from the non-viable cells according to the manufacturer's protocol (BD Pharmingen FITC–Annexin V kit, San Diego, CA) using a benchtop flow cytometer (Accuri C6 flow cytometry, Becton Dickinson). Positive cells were determined and quantified by FACS analysis. The membrane phospholipid PS of apoptotic cells is displaced internally for exposure to the outer leaflet of the membrane. This can be identified by using Annexin V, a PS binding protein (28).

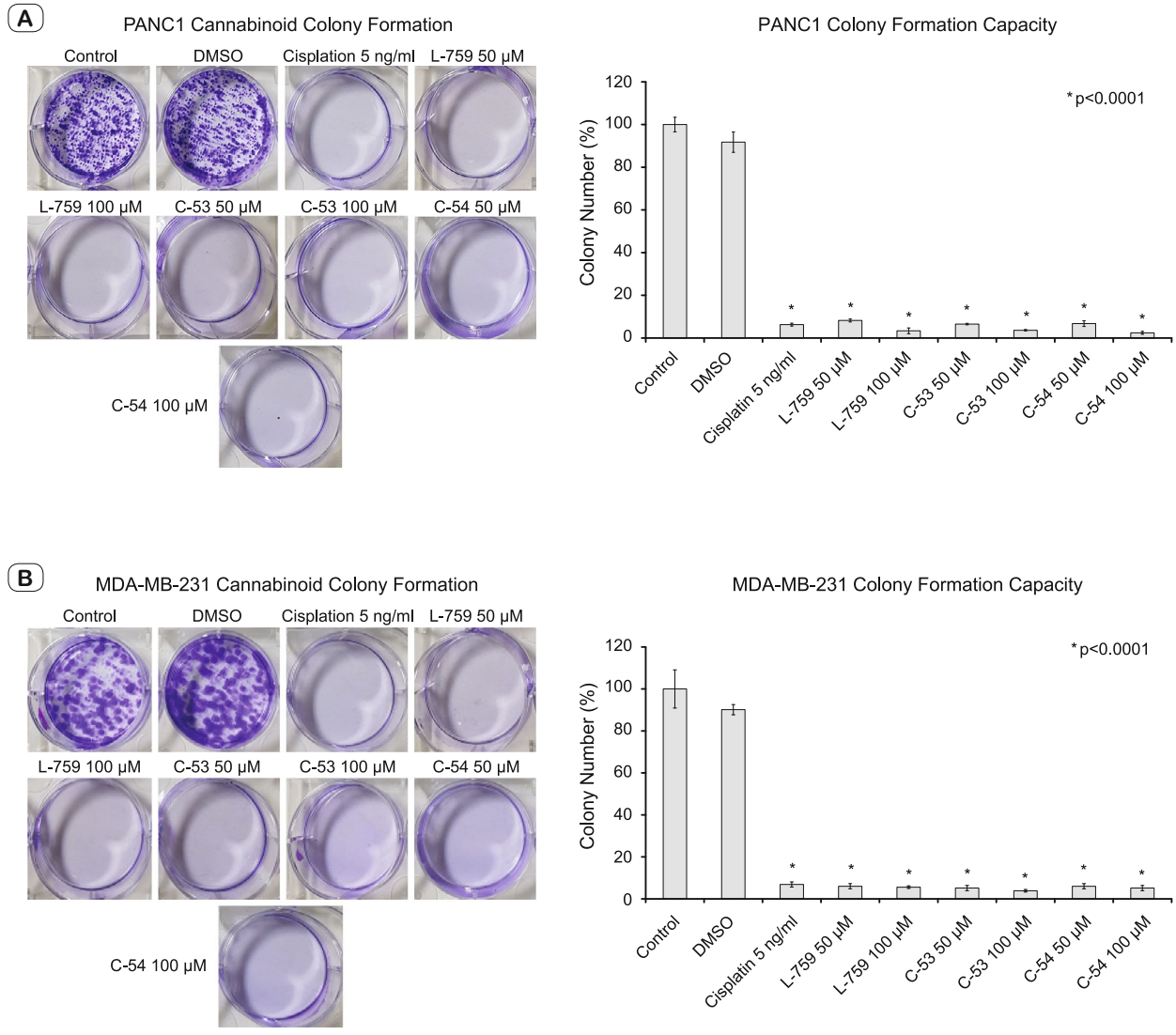

Fig. 2. Effect of increasing concentrations of selective CB2-agonist (L-759633), CB1-agonist-ACPA and CB1-agonist-ACEA agonists on colony formation capacity (A) in human PANC1 pancreatic cancer cells, (B) in human MDA-MB-231 breast cancer cells. Clonogenicity assays were performed by incubating cells with 50 or 100 µM L-759633, ACPA, ACEA or cisplatin 5 ng/ml. Data are expressed as the mean (± SD) values of three independent experiments. * p < 0.0001 compared to the control group.

Statistical analysis

Data were expressed as the means ± standard deviation values, from at least three experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. A p value < 0.05 were considered statistically significant.

Results

Effect on cell viability and proliferation

Considering the results obtained, selective CB2-agonist -L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA applications significantly and dose-dependently decreased cell proliferation in PANC1 cancer cells (* p < 0.005; ** p < 0.0001) (Fig. 1a). Again, selective cannabinoid agonist administration significantly and dose-dependently decreased cell proliferation in MDA-MB-231 breast cancer cells (* p < 0.005; ** p < 0.0001) (Fig. 1b). For ongoing experiments, the dose that inhibits cell proliferation by approximately 50 % and twice of this dose were chosen as 50 and 100 µM.

Effect on cell clonogenicity

The effect on viability of the cannabinoid agonists on human PANC1 pancreatic and MDA-MB-231 breast cancer cells was tested for their influence on clonogenicity. Tests were conducted at 50 and 100 µM for L-759633, ACPA and ACEA in culture plates. All the three cannabinoid agonists strongly inhibited colony formation for human PANC1 pancreatic cell line (* p < 0.0001) (Fig.

2a). Again, selective cannabinoid agonist administration significantly and dose-dependently decreased cell clonogenicity in MDA-MB-231 breast cancer cells (* $p < 0.0001$) (Fig. 2b). The colony counts were graphed to better visualize (Fig. 2).

Western blot analysis

In Western blot analysis, a significant and dose-dependent increase was observed in pro-apoptotic protein Bax levels in PANC1 pancreatic cancer cells with selective CB2-agonist -L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA application. On the contrary, a significant decrease was observed in anti-apoptotic protein Bcl-2 levels (Fig. 3). In Western blot analysis, a significant and dose-dependent increase in proapoptotic protein Bax levels was observed in MDA-MB-231 breast cancer cells with selective cannabinoid agonist applications, on the contrary, a significant decrease was observed in anti-apoptotic protein Bcl-2 levels (Fig. 4).

Flow cytometry analysis

To investigate underlying molecular mechanisms of growth inhibition observed by treatments with CB2-agonist-L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA in breast and pancreatic cancer cells, after 72 h of treatment, we evaluated apoptosis induction through cannabinoid agents, using double staining annexin V / propidium iodide (Fig. 5). AnnexinV +/PI – stained section defines cells in early apoptosis; cells which are stained with only PI (AnnexinV -/PI +) are early necrotic cells, although AnnexinV +/PI + stained part represents the cells in primary necrosis and late apoptosis related with secondary necrosis (28). As shown in Figure 5a, administration of cannabinoid agents triggered apoptosis (as evaluated by induction of the apoptotic cells as positively stained in AnV +/PI - and AnV +/PI + groups, as 17.2 %, 20.3 %, 31.3 %, 98.8 % and 99.5 % in cisplatin 5 ng/ml, L-759 10 µM, L-759 50 µM, ACPA 50 µM and ACEA 50 µM treated groups in PANC1 pancreatic cancer cells, respectively). And as shown in Figure 5b, administration of cannabinoid agents triggered apoptosis (as evaluated by induction of the apoptotic cells as positively stained in AnV +/PI - and AnV +/PI + groups, as 16.7 %, 21.1 %, 32.1 %,

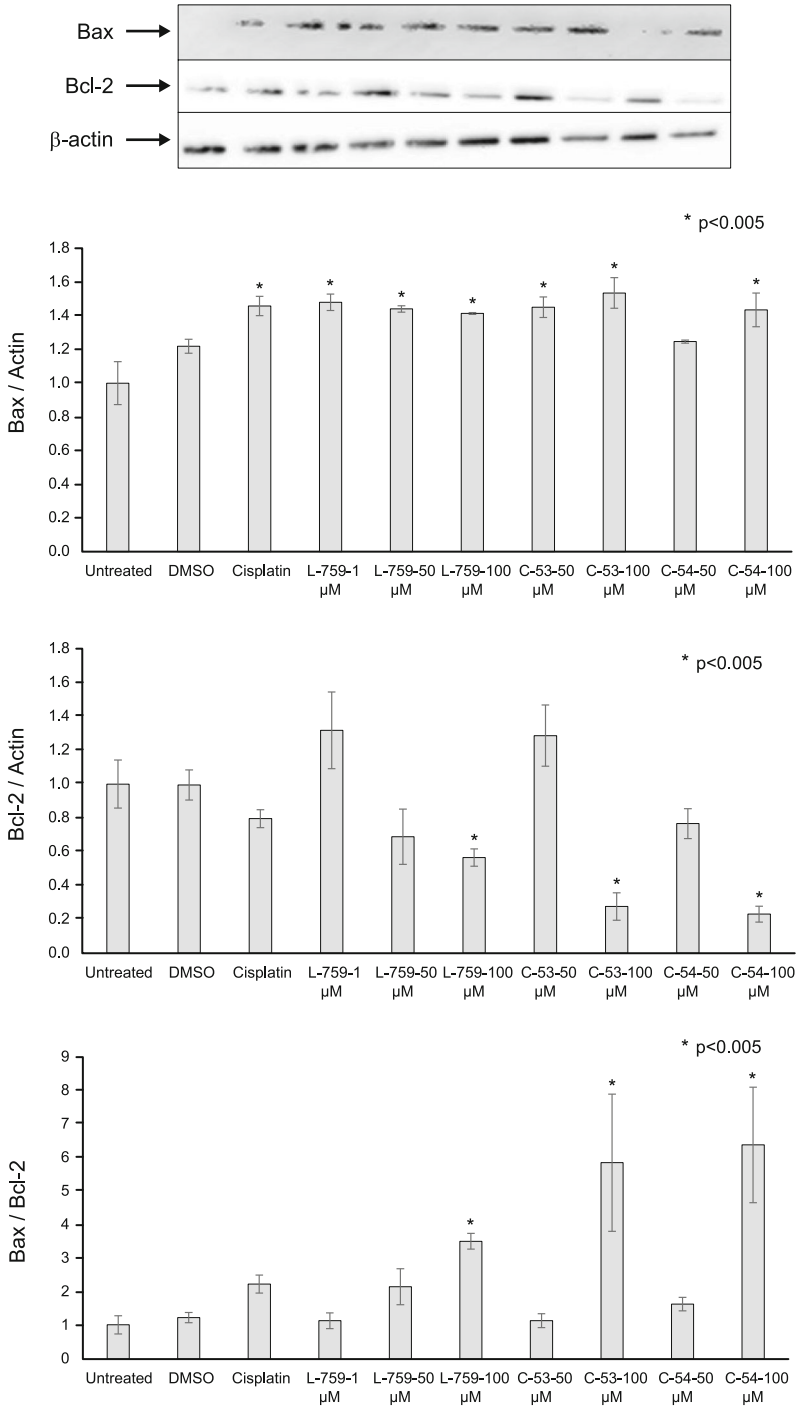

Fig. 3. Western blot analysis for human PANC1 pancreatic cancer cells. The changes in Bax and Bcl-2 protein levels following CB2-agonist (L-759633) (1, 50 and 100 µM), CB1-agonist-ACPA (50, 100 µM) and CB1-agonist-ACEA (50, 100 µM) agonist treatments. The graphs show the relative densitometric values of the indicated proteins. Determination of the amount of protein product was performed by densitometric scanning. Data are normalized using β-actin signal and expressed in arbitrary densitometric units. Values are means SD, * $p < 0.005$ significant difference from untreated cells.

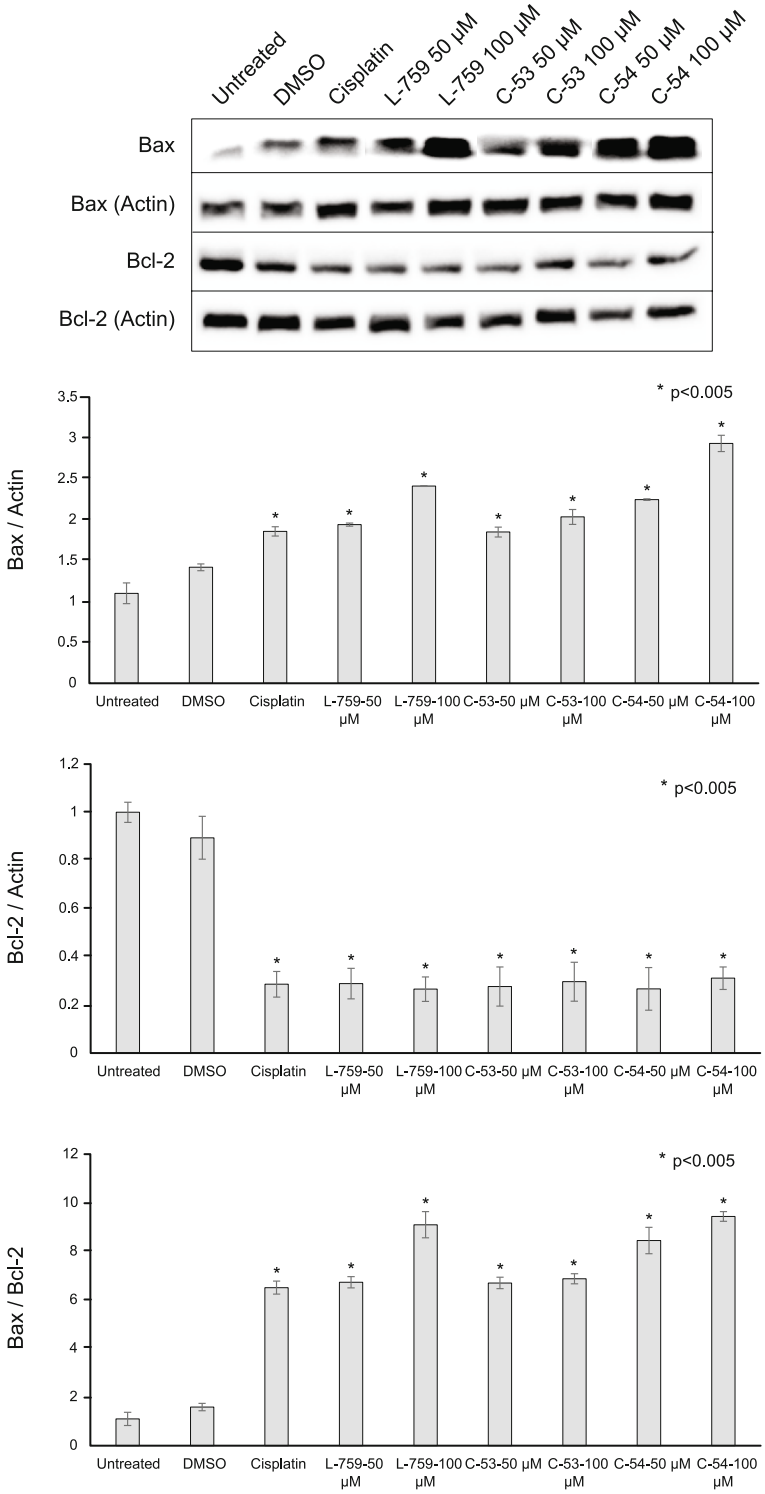

Fig. 4. Western blot analysis for human MDA-MB-231 breast cancer cells. The changes in Bax and Bcl-2 protein levels following CB2-agonist (L-759633) (1, 50 and 100 µM), CB1-agonist-ACPA (50, 100 µM) and CB1-agonist-ACEA (50, 100 µM) agonist treatments. The graphs show the relative densitometric values of the indicated proteins. Determination of the amount of protein product was performed by densitometric scanning. Data are normalized using β-actin signal and expressed in arbitrary densitometric units. Values are means SD, * p < 0.005 significant difference from untreated cells.

55.7 % and 94.6 % in Cisplatin 5ng/ml, L-759 10 µM, L-759 50 µM, ACPA 50 µM and ACEA 50 µM treated groups in MDA-MB-231 cells, respectively.

Discussion

In this study, we investigated the effects of three different selective cannabinoid agonists, selective CB2-agonist -L-759633, CB1-agonist-ACPA and CB1-agonist-ACEA on the cell proliferation, colonogenicity and apoptosis in human PANC1 pancreatic and MDA-MB-231 breast cancer cells. Our results demonstrate that these agents with increasing concentrations exert an intense antiproliferative effect and supress colony formation capacity in both human PANC1 pancreatic and MDA-MB-231 breast cancer cells. Also, our results suggest apoptosis as one of the mechanisms of these agonists to reduce tumour cell survival.

In various cancer types, endo-, phyto- and synthetic cannabinoids have been shown to have antiproliferative, antiangiogenic, proapoptotic and antimetastatic effects due to regulating cellular signalling pathways, which are critical for cell survival and growth (15, 18, 29, 30). More extensive research is needed to determine the full potential of synthetic cannabinoids in cancer. The anti-cancer effects of cannabinoids have been reported in the case of pancreatic cancer (31–34). It has been demonstrated that cannabidiol and tetrahydrocannabinol can suppress pancreatic cancer growth, and that this may be partially through inhibition of p-21 activated kinase 1 (PAK1) (34). Parallel to our study, it has been shown that both CB1 and CB2 receptor agonists act through a widely common mechanism that involves cell growth regulation and apoptosis in pancreatic adenocarcinoma (35). Again, it has been shown that cannabinoids induce apoptosis on pancreatic cancer cells by the activation of the p8-ATF-4-TRB3 proapoptotic pathway (31). Dando et al stated that in pancreatic adenocarcinoma cells, autophagy induction dependent to cannabinoids was related to ROS-dependent increase of the AMP/ATP ratio (32).

Increased expression of CB1 and CB2 receptors has been reported in various breast cancer cell lines and tissues (36). The first

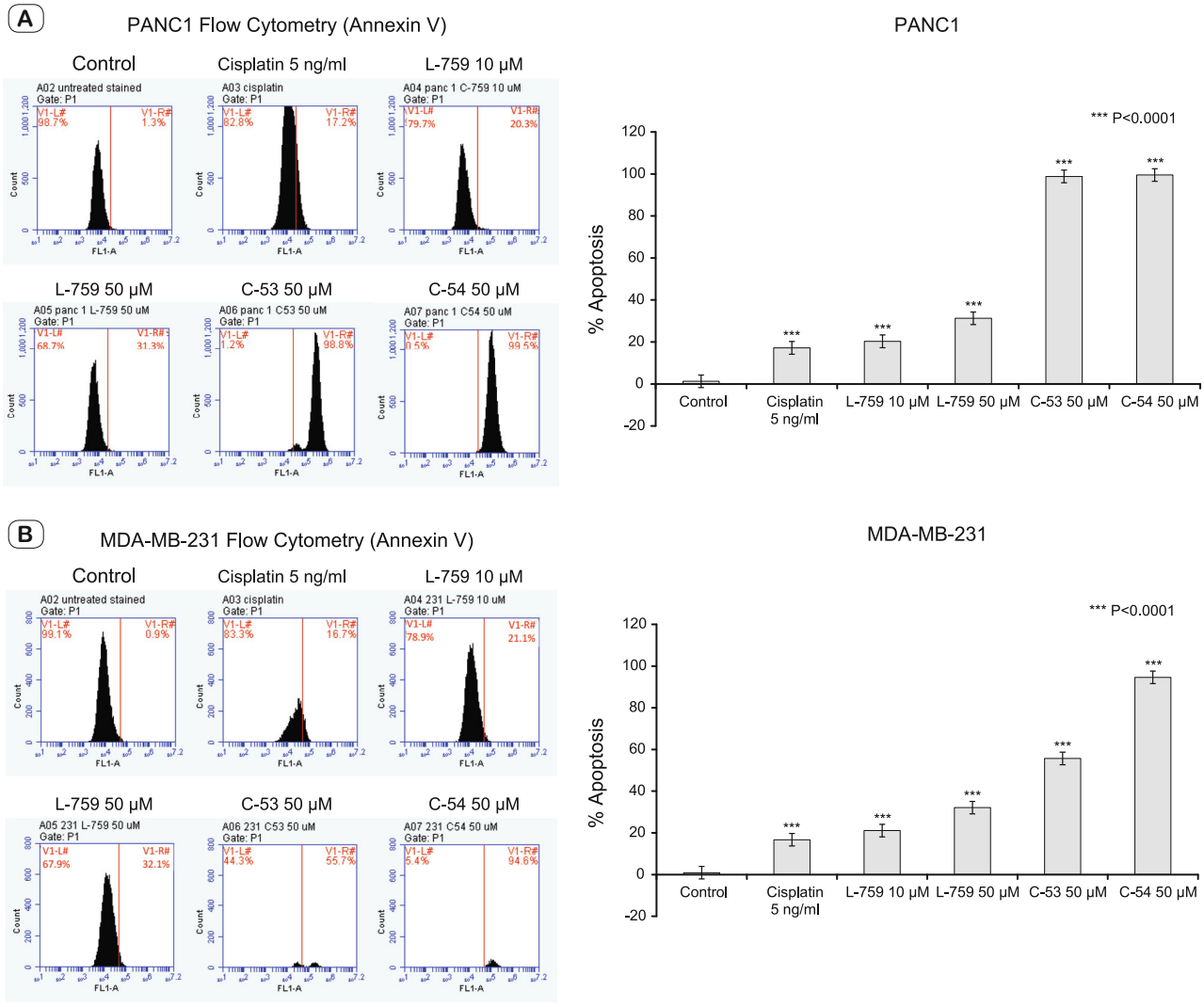

Fig. 5. Flow cytometric analysis of selective CB2-agonist (L-759633) (10, 50 µM), CB1-agonist-ACPA (50 µM), and CB1-agonist-ACEA (50 µM), agonists in human PANC1 pancreatic cancer cells and human MDA-MB-231 cancer cells. Histograms show cell cycle profiles and percentage of apoptosis is shown in bar graphic. Values are means SD, * p < 0.0001 compared with the control group.

identified endogenous ligand for cannabinoid receptor, anandamide has been shown to block breast cancer cell proliferation by CB1 like receptor mediated inhibition of prolactin action (37). Anandamide analogue, 2-methyl-2′-F-anandamide (Met-F-AEA), has been suggested to have the ability to inhibit the invasion of breast cancer cells and that this effect is mediated through the inactivation of the β-catenin (38). Again, CB1 receptor activation has been suggested to be a target for therapeutic strategies to retard the growth of breast carcinoma to inhibit metastatic spreading (30). Selective CB1 receptor agonist, ACEA has been shown to decrease the invasive potential of breast cancer stem cells, demonstrating that CB1 receptor contributes to stem cell properties (39). CB2 receptors are over expressed in breast tumours, and this expression positively correlates with the histological grade. It has been suggested that in breast cancer cells, the apoptosis induced by CB2-selective agonist JWH-015 may be mediated by MAPK/ERK activity (40). It has been previously shown that through activation of CB2 receptors, Delta (9)-tetrahydrocannabinol can reduce cell proliferation and induce apoptosis (36). Again, it has been shown that CB2 activation under in vitro and in vivo conditions suppressed breast cancer through inhibiting EGFR/IGF-IR signalling pathways and it has been stated that CB2 might be an important target in breast cancer subtypes (41). According to the type of cannabinoid and the model used, the proposed mechanisms for the regulation of proliferation of cancer cells differ in literature. Consistent with our study it has been observed that synthetic agonists JWH-133 (CB2 agonist) and WIN-55,212-2 (CB1 and CB2 agonist) inhibited cell proliferation, migration and induced apoptosis in breast cancer cell lines in vitro (42). Again, mixed CB1/CB2 agonist CP55 940 (43) have been shown to inhibit cell proliferation in breast cancer cells. Tamoxifen has been shown to act as an inverse agonist for CB1/2 receptors via modulating adenylate cyclase activity and to

lead to increases in intracellular cAMP (44). In the current study, we showed that CB2 agonist -L-759633, CB1 agonist-ACPA and CB1agonist-ACEA exerted a marked cytotoxic effect against human PANC1 pancreatic and MDA-MB-231 breast cancer cells with a potency comparable with that observed for cisplatin. The results of MTS assay on human PANC1 pancreatic cancer cells in the presence of selective cannabinoid agonists illustrated that these agonists increased cell proliferation. In particular, we showed that selective cannabinoid agonists, CB2 agonist -L-759633 (50, 100, 250 μM), CB1 agonist-ACPA (50, 100, 250 μM) and CB1 agonist-ACEA (100, 250 μM) were capable of eliciting cytotoxic effects in MDA-MB-231 breast cancer cells. Performed MTS assay confirmed this data, clearly showing a significant anti-proliferative effect in a dose dependent manner.

To further evaluate the effect of cannabinoid receptor agonism on human PANC1 pancreatic cells and MDA-MB-231 breast cancer cell lines, clonogenicity assay was performed with treatment with L-759633, ACPA and ACEA. All the three agonists caused a decreased colony formation in both cell lines dose dependently. Colony formation from cell assemblages may be associated with cell-cell adhesion and cell motility (45) therefore, suggesting that in the presence of CB1/2 agonist administration, both PANC1 pancreatic cells and MDA-MB-231 breast cancer cells are less mobile and more adherent to each other.

In cancer treatment, as a general rule, controlling unwanted cellular toxicity, weakening the proliferation of cancer cells and overcoming intrinsic drug resistance is important. For this purpose, induction of apoptosis is an ideal approach for a selective killing of the cancer cells. Apoptosis, programmed cell death, is responsible for the eradication of damaged cells and has an important role in regulating cell proliferation, balancing cell survival and death. Intrinsic (mitochondrial dependent) and extrinsic (receptor mediated) pathways activate caspase 3 and result in apoptosis (46). Caspase 3 and 9 are activated when proapoptotic (i.e. Bak and Bax) and antiapoptotic (i.e. Mcl-1, Bcl-2 and Bcl-xL) proteins stabilize the membrane of mitochondria (47). Cancer cells regulate this process and reduce apoptosis resulting in elevated drug resistance. We evaluated Bcl-2 and Bax, in order to investigate in more details the underlying mechanism of selective agonists. Bax and Bcl-2 with opposite effects are two members of the Bcl-2 family. Bcl-2, is found at high levels of many human tumours and neutralizes the proapoptotic effect of BAX by forming a heterodimer with Bax (48, 49). In our study, after treatment of PANC1 pancreatic and MDA-MB-231 breast cancer cells with cannabinoid agonist the proapoptotic/antiapoptotic balance drifted to the proapoptotic side. CB2 agonist -L-759633, CB1 agonist-ACPA and CB1 agonist-ACEA decreased the expression of antiapoptotic Bcl-2 and increased the proapoptotic Bax levels. The results were confirmed by flow cytometry. An increase in apoptotic cells was seen after treatment with selective cannabinoid agonists, CB2 agonist -L-759633 (10–50 μM), CB1 agonist-ACPA (50 μM) and CB1 agonist-ACEA (50 μM) both in human PANC1 pancreatic and MDA-MB-231 breast cancer cells. The cells showed a clear positivity of the Annexin V test. As shown by western blot and flow cytometric analysis, selective cannabinoid agonists, CB2 agonist -L-759633, CB1 agonist-ACPA and CB1 agonist-ACEA are good apoptosis inducers in human PANC1 pancreatic and MDA-MB-231 breast cancer cells.

Identifying effective treatments is critical for managing and improving cancer treatment. Inhibiting cannabinoid receptors selectively produces a potential for the therapy of various cancers, including pancreatic and breast cancers. The results presented here show that selective cannabinoid agonists, CB2 agonist -L-759633, CB1 agonist-ACPA and CB1agonist-ACEA supress cell proliferation, clonogenicity and induce apoptosis in human PANC1 pancreatic and MDA-MB-231 breast cancer cells in vitro. We observed that both CB1 and CB2 agonists act through a mechanism that involves up- and down regulations of proteins, which are related to cell growth regulation and energetic metabolism. This evidence suggests a link between the inhibition of cell survival and proapoptotic activity of selective CB1/2 receptor agonists considering as novel pharmacological anti-cancer agents. Overall, we think that our results may contribute to the development of cannabinoid-based therapy in the prevention and management of pancreatic and breast cancers. However, further studies investigating specific mechanisms and the molecular pathways associated with cannabinoid activities are needed.

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