

Cannabinoids Protect Cells from Oxidative Cell Death: A Receptor-Independent Mechanism¹

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ABSTRACT

Serum is required for the survival and growth of most animal cells. In serum-free medium, B lymphoblastoid cells and fibroblasts die after 2 days. We report that submicromolar concentrations of Δ^9 -tetrahydrocannabinol (THC), Δ^8 -THC, cannabinal, or cannabidiol, but not WIN 55,212-2, prevented serum-deprived cell death. Δ^9 -THC also synergized with platelet-derived growth factor in activating resting NIH 3T3 fibroblasts. The cannabinoids' growth supportive effect did not correlate with their ability to bind to known cannabinoid receptors and

showed no stereoselectivity, suggesting a nonreceptor-mediated pathway. Direct measurement of oxidative stress revealed that cannabinoids prevented serum-deprived cell death by antioxidation. The antioxidative property of cannabinoids was confirmed by their ability to antagonize oxidative stress and consequent cell death induced by the retinoid anhydroretinol. Therefore, cannabinoids act as antioxidants to modulate cell survival and growth of B lymphocytes and fibroblasts.

Marijuana has been known for centuries to be a psychoactive medicinal plant (Nahas, 1984). Among its >60 different cannabinoids, (–)- Δ^9 -tetrahydrocannabinol (THC) and cannabidiol are most abundant (Turner et al., 1980). (–)- Δ^9 -THC is the most potent psychoactive compound in marijuana and cannabidiol is nonpsychoactive (Dewey, 1986). In recent years, two cannabinoid receptors, CB1 and CB2, have been identified as G protein-coupled 7-transmembrane-spanning receptor proteins (Matsuda et al., 1990; Munro et al., 1993). CB1, preferentially expressed in brain, mediates the psychoactivity of cannabinoids. CB2 is highly expressed in immune cells; however, its biological functions have yet to be determined. There are numerous, sometimes contradictory, reports of cannabinoid effects on proliferation and cytolysis of T cells, proliferation and antibody production of B cells, nitric oxide (NO) release by macrophages, and cytolysis of natural killer cells (Thomas et al., 1998).

During metabolic cellular processes, oxidative species such as superoxide radical anion, hydrogen peroxide, and lipid peroxides are generated intracellularly (Scandalios, 1997). These oxidative species, if not eliminated, damage DNA, protein, or membrane lipids and cause oxidative cell death. Thus, endogenous antioxidative enzymes such as superoxide dismutase, catalase, and peroxidase, as well as endogenous

small-molecule antioxidants such as vitamin E, vitamin C, and ubiquinol are required for cells to survive (Scandalios, 1997). Exogenous small-molecule antioxidants also have been shown to effectively prevent oxidative cell death in cultured cells (Busciglio and Yankner, 1995; Johnson et al., 1996; Nakao et al., 1996; Hampson et al., 1998). In this report, we study the mechanism whereby cannabinoids affect cultured human B lymphoblastoid cells and mouse fibroblasts.

Materials and Methods

Reagents and Cells. (–) and (+)- Δ^9 -THC, (–)- Δ^8 -THC were provided by the National Institute on Drug Abuse (Rockville, MD); cannabinal, cannabidiol, insulin, holo-transferrin, and delipidated BSA were purchased from Sigma (St. Louis, MO); WIN 55,212-2 was obtained from Research Biochemicals (Natick, MA); anhydroretinol was a generous gift from Dr. Fadila Derguini (Institut De Recherche P. Fabre, Toulouse, France); 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, di(acetoxymethyl ester) is a product of Molecular Probes Inc. (Eugene, OR); [³H]thymidine (6.7 Ci/mmol) was purchased from DuPont/NEN (Boston, MA), and recombinant human platelet-derived growth factor (PDGF) B/B and WST-1 from Boehringer Mannheim (Indianapolis, IN). RNAsol B was purchased from Biotecx Laboratories, Inc. (Houston, TX); Superscript reverse transcriptase from Life Technologies (Grand Island, NY); and KlenTaq enzyme from Clontech (Palo Alto, CA). The Epstein-Barr virus-transformed human B lymphoblastoid cell line 5/2 was established from the blood of a healthy volunteer according to a method de-

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ABBREVIATIONS: THC, tetrahydrocannabinol; DMEM, Dulbecco's modified Eagle's medium; CB, cannabinoid receptor; NO, nitric oxide; PDGF, platelet-derived growth factor; ITLB, insulin-, transferrin-, linoleic acid-, and BSA-containing medium; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair.

scribed in detail in Emanuel et al. (1984). NIH 3T3 cells were purchased from the American Type Culture Collection (Rockville, MD). The 5/2 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 100 U/ml penicillin/streptomycin. NIH 3T3 cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum. Serum-free ITLB medium used was RPMI-1640 medium supplemented with 5 μ g/ml insulin, 5 μ g/ml holo-transferrin, 20 μ M linoleic acid, and 0.1% delipidated BSA.

Measurement of Cell Proliferation by [3 H]Thymidine Incorporation. Cultured 5/2 cells were washed once and plated into 96-well microtiter plates in ITLB medium (100 μ l/well), at a cell density of 100,000 cells/ml. One day later, reagents dissolved in 100 μ l of ITLB medium were added to each well. After incubation for 2 days, cells were labeled with [3 H]thymidine (1 μ Ci/well). After 5 h, cells were harvested on glass fiber filtermats.

To quantify the number of cycling NIH 3T3 cells, a suspension of 5000 cells in 100 μ l of DMEM containing 10% calf serum was plated into each well, allowed to grow to near confluency, and then growth was arrested in 150 μ l/well of DMEM containing 0.5% calf serum. After 2 days, cells were treated with reagents in 200 μ l/well of serum-free RPMI-1640 medium containing 0.1% BSA for 24 h and labeled with [3 H]thymidine (1 μ Ci/well) for the last 13 h.

Measurement of Cell Viability by WST-1. WST-1 was used according to the manufacturer's instructions. WST-1 measures cellular mitochondrial respiratory activity. NIH 3T3 cells in 100 μ l of serum-containing medium were plated at a density of 80,000 cells/ml, and allowed to grow to 80% confluency. After growth arrest for 2 days in 0.5% calf serum, 3T3 cells were treated with reagents in RPMI-1640 medium containing 0.1% BSA. At the end of 2-day incubation, WST-1 (13 μ l/well) was pulsed for 2 h. The absorbance ($A_{450\text{ nm}} - A_{650\text{ nm}}$) was determined with a 96-well reader (Molecular Dynamics, Sunnyvale, CA).

Determining the Expression of Cannabinoid Receptors on 5/2 and NIH 3T3 Cells by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Total RNA was isolated from cultured cells with RNazol B according to the manufacturer's protocol. After treatment with RNase-free DNase, RNA was reverse transcribed into first-strand cDNA with Superscript reverse transcriptase. The quality of first-strand cDNA was confirmed by PCR with β -actin primers. The oligonucleotide sequences used in all primers were conserved between human and mouse. CB1 primers were 5'-ggccttgcagataccacttccg-3' (sense) and 5'-atgaagtggtaggaaggcctgca-3' (antisense). CB2 primers were 5'-atgaccttcacagcctctgtggg-3' (sense) and 5'-ggcactgcctgtcctgtgtg-3' (antisense). β -Actin primers were 5'-gacccagatcatgtttgagacc-3' (sense) and 5'-ggcctcaggaggagaatgatc-3' (antisense). The expected size for human CB1 is 449 base pairs (bp), for mouse CB1, 452 bp; for human and mouse CB2, 353 bp; and for human and mouse actin, 649 bp. PCR conditions for CB1 and CB2 were 4 min initial denaturing at 94°C, 35 cycles of 30 s at 94°C and 2 min at 72°C, followed by 7-min extension at 72°C. PCR for β -actin was 5 min initial denaturing at 94°C, 35 cycles of 30 s at 94°C and 30 s at 62°C and 1 min at 72°C, followed by 7-min extension at 72°C. KlenTaq enzyme was used in all PCRs. Mouse brain first-strand cDNA was used as positive control for CB1 (Matsuda et al., 1990), and Jurkat cDNA for CB2 (Schatz et al., 1997). A 50- to 2000-bp ladder (Ampli-size molecular ruler; Bio-Rad, Richmond, CA) was used as molecular marker.

Flow Cytometry Analysis of Cellular Oxidative Stress and Cell Viability. Oxidative stress in living cells was directly measured by flow cytometry with 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, di(acetoxymethyl ester), a membrane-permeable and oxidation-sensitive reagent (Hockenbery et al., 1993). The acetate and acetoxymethyl ester groups of this reagent are enzymatically cleaved in the cell. After oxidation by cellular reactive oxygen species, the resulting fluorescent product is retained inside living cells due to its electric charges and emits light with an intensity proportional to the level of cellular oxidative stress. Flow cytometry was used to mea-

sure simultaneously cellular oxidative stress by fluorescence and cell viability by side and forward scatterings, thus providing a direct correlation between oxidative stress and cell death in the same sample.

The 5/2 cells, at a density of 80,000 to 100,000 cells/ml, were cultured in ITLB medium alone or with reagents. At given times, cells were treated with 5 μ M 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate, di(acetoxymethyl ester) for 1 h, followed by fluorescence-activated cell-sorting analysis (FACSCalibur; Becton Dickinson and Company, Franklin Lakes, NJ). For fluorescence measurement, the excitation and emission wavelengths were set at 488 and 530 nm, respectively. For each sample, 5000 to 6000 cells were analyzed. Fluorescence intensity was quantified with CellQuest software.

Results

Cannabinoids Prevented Serum-Deprived Cell Death of Lymphoblastoid Cells in Serum-Free Medium. The Epstein-Barr virus-transformed human B lymphoblastoid 5/2 cells grow indefinitely in tissue culture medium containing fetal bovine serum. However, if cultured in serum-free defined ITLB medium, they die after 2 days (Buck et al., 1990). Addition of cannabinoids prevented this serum-deprived cell death. As shown in Fig. 1A, (-)- Δ^9 -THC and (+)- Δ^9 -THC replaced serum and supported the growth of lymphoblastoid cells cultured in ITLB medium. Submicromolar concentrations of (-)- Δ^8 -THC, cannabinal, or canna-

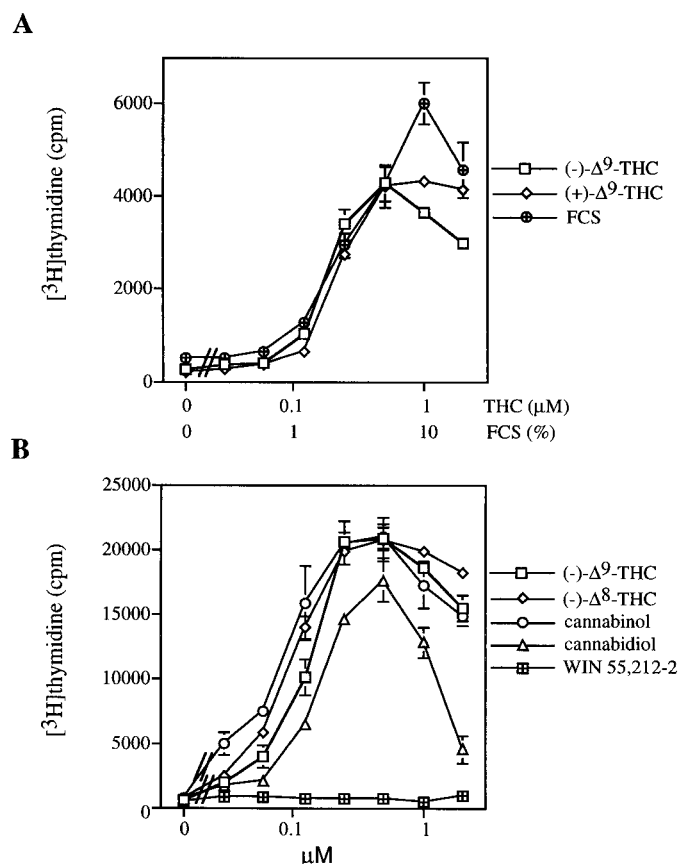


Fig. 1. Cannabinoids increased the number of cycling lymphoblastoid 5/2 cells under serum-deprived conditions. A, dose-response curves of (-)- Δ^9 -THC, (+)- Δ^9 -THC, and fetal calf serum (FCS). B, dose-response curves of different cannabinoids. Cell numbers in A and B measured by [3 H]thymidine incorporation. Data represent the mean $\pm$ S.D. of triplicate measurements.

bidol also were active, whereas the synthetic cannabinoid agonist WIN 55,212-2, was inert (Fig. 1B). Cannabinoids displayed a narrow effective concentration range. $(-)\Delta^9$ -THC, $(-)\Delta^8$ -THC, and cannabidiol were toxic to cells at $\geq 5 \mu\text{M}$. Cannabidiol started to be toxic at $\geq 1 \mu\text{M}$.

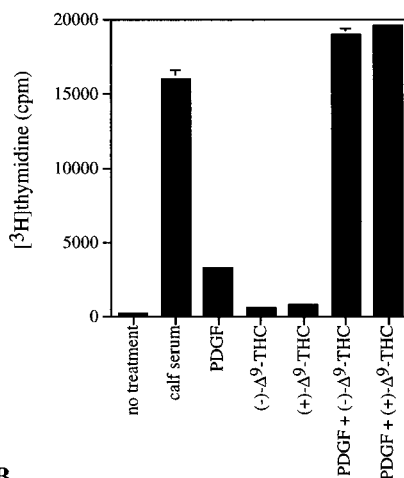
The cannabinoids' cell survival activity did not reflect their binding affinity to the CB1 and CB2 receptors expressed on 5/2 cells (see RT-PCR results below). The K_d value of CB1 and CB2 binding is $\sim 10^{-9}$ M for WIN 55,212-2, 10^{-8} M for $(-)\Delta^9$ -THC and $(-)\Delta^8$ -THC, 10^{-7} M for cannabidiol, and 10^{-6} M for cannabidiol (Showalter et al., 1996). In the cell survival assay, however, the high-affinity ligand WIN 55,212-2 was inert; the low-affinity ligand cannabidiol was bioactive; and the concentration required for cell survival activity was >10 times higher than that required for the receptor binding for $(-)\Delta^9$ -THC and $(-)\Delta^8$ -THC, or 10 times lower for cannabidiol (Fig. 1B). In addition, contrary to the receptor-binding affinity, cannabinoids' cell survival activity did not show stereoselectivity because naturally occurring $(-)\Delta^9$ -THC and its synthetic enantiomer $(+)\Delta^9$ -THC were equally bioactive (Fig. 1A). The poor correlation between cell survival activity and CB1/CB2 receptor-binding affinity and the lack of stereoselectivity suggest a nonreceptor-mediated pathway.

Cannabinoids Prevented Serum-Deprived Cell Death in NIH 3T3 Cells and Synergized with PDGF in Activating Resting NIH 3T3 Cells. When 3T3 cells are cultured in DMEM containing 0.5% calf serum, they arrest in a quiescent, nondividing state within 1 day (Dulbecco, 1970). One-day starved cells can be activated to reenter cell cycle by either PDGF or serum; however, those starved >1 day undergo cell death with or without PDGF, thus they can only be activated by serum or a combination of PDGF and retinol (Chen et al., 1997). As measured by $[^3\text{H}]$ thymidine incorporation, cannabinoids were not mitogenic, but they greatly potentiated PDGF activation in resting cells from 2-day serum-starvation (Fig. 2A). The combination of PDGF and cannabinoids reached the same activation level achieved by serum. As measured by WST-1, addition of submicromolar $(-)\Delta^9$ -THC prevented serum-deprived cell death (Fig. 2B). PDGF increased the cell survival activity of $(-)\Delta^9$ -THC because the effective concentration of $(-)\Delta^9$ -THC is lower in its presence (Fig. 2B). Among the cannabinoids tested, the relative potency and dose dependence of PDGF synergistic effect in NIH 3T3 cells (Fig. 2C) were very similar to that of growth supportive effect demonstrated in the B-lymphoblastoid cell proliferation assays (Fig. 1), suggesting a common mechanism of cannabinoid action on these cells.

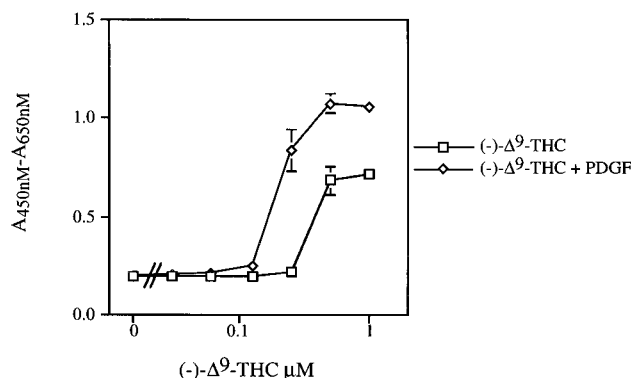
Cannabinoids' Cell Survival Activity Was Independent of Known Cannabinoid Receptors. Expression of the known cannabinoid receptors CB1 and CB2 in 5/2 and NIH 3T3 cells were tested by RT-PCR (Fig. 3). Human B lymphoblastoid 5/2 cells expressed low amounts of CB1 mRNA and high amounts of CB2 mRNA. Expression of CB1 and CB2 has been reported in the human B-lymphoblastoid cell line Daudi (Bouaboula et al., 1993; Galiege et al., 1995). NIH 3T3 cells expressed neither CB1 nor CB2. The lack of expression of known cannabinoid receptors in 3T3 cells supports the notion that the observed cell survival or growth supportive effect of cannabinoids is a nonreceptor-mediated process.

Reduction of Cellular Oxidative Stress by Cannabinoids Correlated with Prevention of Serum-Deprived

A



B



C

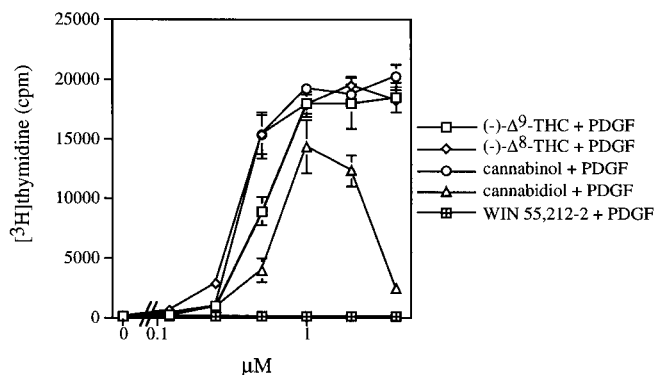


Fig. 2. Cannabinoids prevented serum-deprived cell death in NIH 3T3 cells and synergized with PDGF in activating 3T3 cells arrested by serum-starvation. A, activation of 2-day serum-starved 3T3 cells by calf serum (20%), PDGF (50 ng/ml), $(-)\Delta^9$ -THC (0.5 μM), $(+)\Delta^9$ -THC (0.5 μM), or the combination of $(-)$ or $(+)\Delta^9$ -THC with PDGF. B, $(-)\Delta^9$ -THC prevented serum-deprived cell death in the absence or presence of PDGF (50 ng/ml). C, dose-response curves of cannabinoids in combination with PDGF (50 ng/ml). Activation in A and C were measured by $[^3\text{H}]$ thymidine incorporation. Cell viability in B was measured by WST-1. In all figures, data represent the mean $\pm$ S.D. of triplicate measurements.

Cell Death. Human B lymphoblastoid 5/2 cells were cultured in serum-free ITLB medium alone or in the presence of 0.5 μM $(-)\Delta^9$ -THC or 0.2 μM α -tocopherol, the most potent isomer of the antioxidant vitamin E. Cellular oxidative stress and cell viability were simultaneously recorded for 3 days (Fig. 4). Cellular oxidative stress was measured by fluorescence and cell viability by forward and side scatterings. On

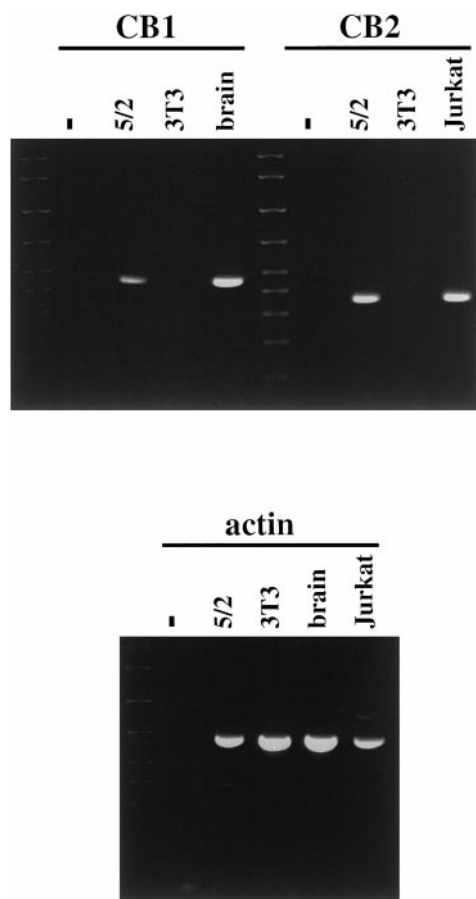


Fig. 3. Human B lymphoblastoid 5/2 cells expressed CB1 and CB2; NIH 3T3 fibroblasts expressed neither CB1 nor CB2. CB1 and CB2 gene expression was measured by RT-PCR. First-strand cDNAs of mouse brain and human Jurkat cells served as positive control of PCRs for CB1 and CB2, respectively; absence of first-strand cDNA was negative control. Quality of first-strand cDNA was confirmed with primers for β -actin. The 50- to 2000-bp ladder was used as a marker. Data represent one of two independent measurements.

day 1, the histogram peak in the untreated sample shifted rightward compared with the treated ones, reflecting a near 2-fold increase of cellular oxidative stress in the untreated sample (Fig. 4B, day 1, left). The scattering patterns in the dot-plots showed no difference in cell viability and cells were healthy (Fig. 4B, day 1, right). There was a small number of dead cells, distinguished by their weaker forward but stronger side scattering, caused by handling during sample preparation. On day 2, the untreated cells showed 6-fold increase in cellular oxidative stress (Fig. 4B, day 2, left top) and significant amount of cell death (Fig. 4B, day 2, right top). On day three, all untreated cells were dead (Fig. 4B, day 3, right top). In contrast, cells treated with either $(-)\Delta^9$ -THC or α -tocopherol lacked signs of oxidative stress and remained healthy during this period (Fig. 4B). Thus, like the prototypical antioxidant α -tocopherol, $(-)\Delta^9$ -THC was able to reduce the cellular oxidative stress and consequently prevent oxidative cell death in cells cultured under serum-free condition.

$(-)\Delta^9$ -THC Reduced Cellular Oxidative Stress and Cell Death Induced by Retinoid Anhydroretinol. Anhydroretinol, a physiological vitamin A metabolite (Buck et al., 1993; Grün et al., 1997), up-regulates cellular oxidative stress and induces oxidative cell death in 5/2 and NIH 3T3

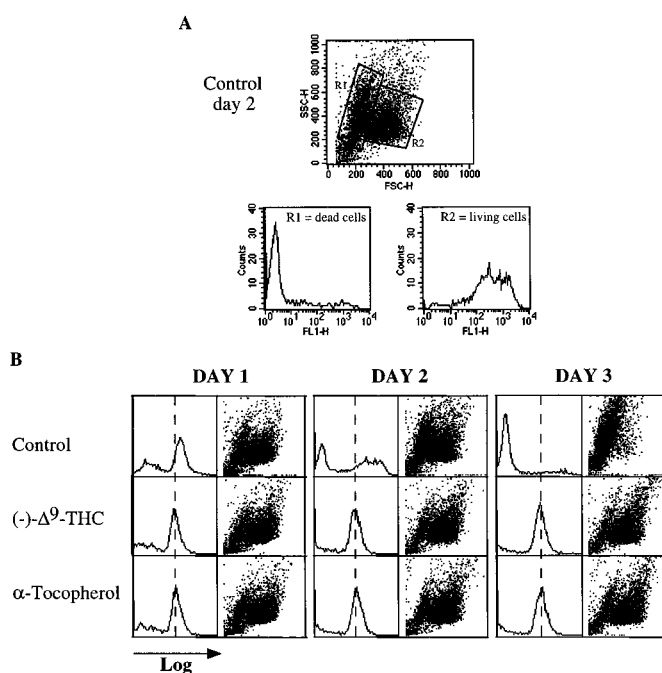


Fig. 4. Reduction of oxidative stress by $(-)\Delta^9$ -THC correlated with its prevention of serum-deprived cell death in 5/2 cells. Cellular oxidative stress and cell viability were simultaneously recorded and displayed side by side. Cellular oxidative stress was measured by fluorescence intensity in histograms and cell viability by forward and side scattering intensities in dot-plots. In the histogram, the x-axis was the relative fluorescence intensity in log scale (FL1-H); the y-axis corresponded to the number of cells (counts). In the dot-plot, the x-axis was the forward scattering intensity (FSC-H), the y-axis the side scattering intensity (SSC-H). A, fluorescence and scattering pattern of living and dead cells after serum-deprivation for 2 days. Living cells gated in R2 had brighter fluorescence and stronger forward but weaker side scatterings than dead cells gated in R1. Loss of membrane integrity in dead cells causes fluorescent dye to diffuse out of the cells; therefore, the cellular fluorescence intensity in dead cells is lower than in living cells. B, time course of oxidative stress and cell viability of 5/2 cells cultured in serum-free medium with or without $(-)\Delta^9$ -THC (0.5 μ M) or α -tocopherol (0.2 μ M). Data represent one of two independent measurements.

cells in both serum-free and serum-containing media (Chen et al., 1997, 1999). We tested whether $(-)\Delta^9$ -THC antagonizes the effect of anhydroretinol. The 5/2 cells were treated with 4 μ M anhydroretinol in the presence of different concentrations of $(-)\Delta^9$ -THC, and cellular oxidative stress (Fig. 5, A and B) and cell viability (Fig. 5A) were followed by fluorescence activated cell-sorting analysis at 1, 3, and 6 h post-treatment. After 1 h, cells showed no significant up-regulation of oxidative stress (Fig. 5, A and B) and were healthy (Fig. 5A). After 3 h, anhydroretinol-treated cells showed 3-fold increase in cellular oxidative stress compared with the untreated cells, and addition of $(-)\Delta^9$ -THC decreased this oxidative stress in a dose-dependent manner (Fig. 5, A and B). Cell death was not detectable at this time point (Fig. 5A). After 6 h, >90% of the cells treated with anhydroretinol alone were dead; cotreatment with 2 μ M Δ^9 -THC decreased the number of dead cells to ~40% (Fig. 5A). The reduction of oxidative stress by $(-)\Delta^9$ -THC correlated well with the suppression of cell death in anhydroretinol-treated cells (Fig. 5, A and B). The dose-dependent prevention of anhydroretinol-induced cell death by $(-)\Delta^9$ -THC in 5/2 cells also was observed by [3 H]thymidine incorporation assay (Fig. 5C).

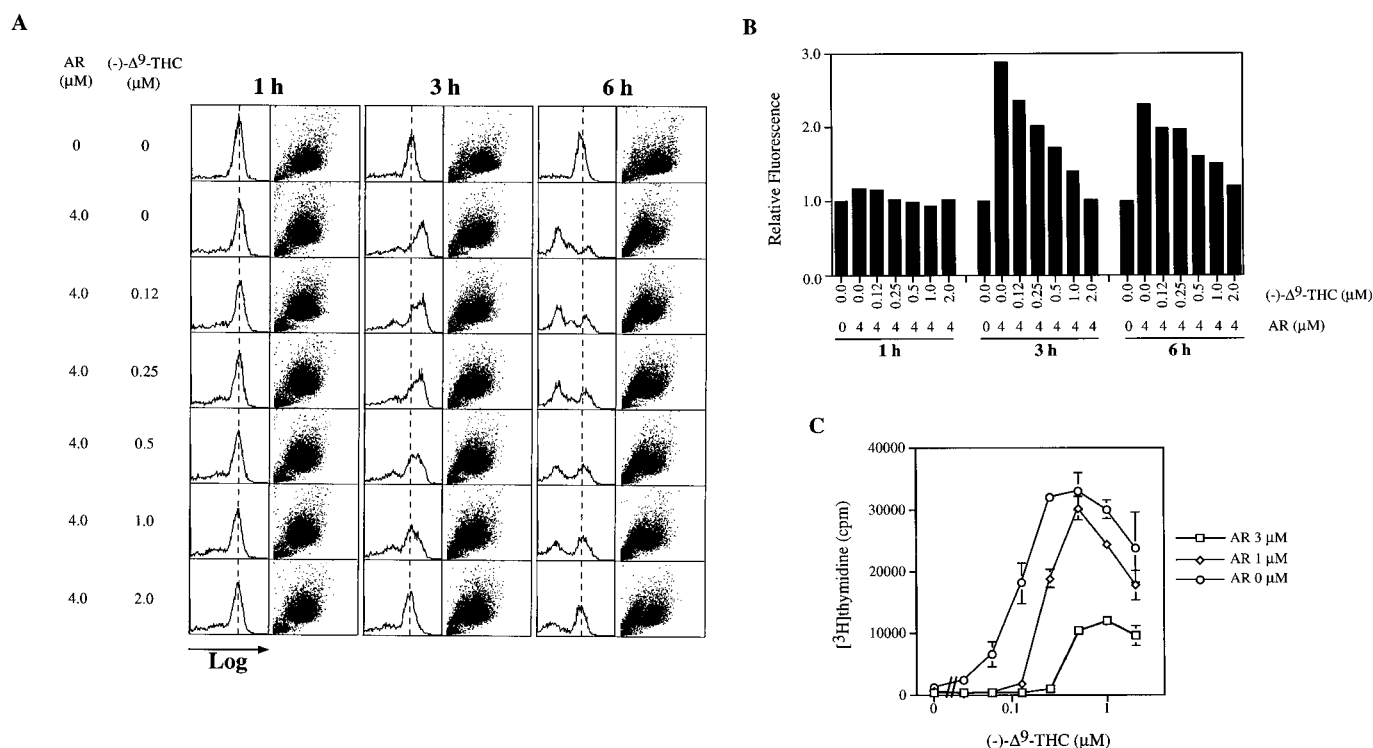


Fig. 5. $(-)\text{-}\Delta^9\text{-THC}$ reduced oxidative stress and cell death induced by anhydroretinol. **A**, flow cytometry measurement of oxidative stress and cell viability in 5/2 cells treated with 4 μM anhydroretinol and different concentrations of $(-)\text{-}\Delta^9\text{-THC}$. Data represent one of two independent measurements. **B**, quantitative presentation of relative cellular oxidative stress shown in **A**. **C**, dose-response curves of $(-)\text{-}\Delta^9\text{-THC}$ against cell death induced by anhydroretinol in 5/2 cells measured by [^3H]thymidine incorporation. Data represent the mean $\pm$ S.D. of triplicate measurements.

Discussion

In this study, we showed that $(-)\text{-}\Delta^9\text{-THC}$, $(-)\text{-}\Delta^8\text{-THC}$, cannabinol, or cannabidiol at submicromolar concentrations prevented serum-deprived cell death of human B lymphoblastoid cells and mouse fibroblast cells. The cannabinoids' growth supportive effect did not correlate with their ability to bind to known cannabinoid receptors and showed no stereoselectivity, suggesting a nonreceptor-mediated pathway. Direct measurement with flow cytometry revealed that cannabinoids prevented cell death by antioxidation. The antioxidative property of cannabinoids was supported by the same action of cannabinoids and α -tocopherol in our assays and by the ability of cannabinoids to antagonize the oxidative stress and consequent cell death induced by anhydroretinol. Our results expand on the knowledge that the antioxidative effect of $(-)\text{-}\Delta^9\text{-THC}$ and cannabinol protects cultured rat cortical neurons from glutamate induced excitatory cell death (Hampson et al., 1998).

The observed cannabinoids' effect on 5/2 and NIH 3T3 cells may be attributed to their antioxidative property. Human lymphoblastoid 5/2 cells, on Epstein-Barr virus transformation, no longer require cytokines for growth; thus, supplementation with antioxidants alone in serum-free ITLB medium is sufficient to maintain cell growth. However, NIH 3T3 cells arrested by serum-starvation still require growth factors such as PDGF to grow. [^3H]Thymidine incorporation quantifies the total number of cycling cells; therefore, it measures both the degree of activation and the survival of activated cells. Our data suggest that cannabinoids may act as antioxidants to prevent oxidative cell death of activated cells occurring under serum-free conditions without directly promoting cell activation.

Cannabinoids' antioxidative properties also may explain the conflicting reports about NO release on treatment with cannabinoids in cultured mammalian macrophages (Coffey et al., 1996; Jeon et al., 1996; Stefano et al., 1996). Cannabinoids increase NO release by coupling to their receptors (Stefano et al., 1996). But at higher concentrations antioxidation by cannabinoids dominates, leading to a decrease in NO release by inhibition of the redox-sensitive nuclear factor- κB activation, which is required for the expression of NO-producing enzyme inducible NO synthase (Coffey et al., 1996; Jeon et al., 1996).

The potency of the antioxidative activity of naturally occurring and synthetic cannabinoids in our assay agrees with that predicated from their chemical structures (Hampson et al., 1998). $(-)\text{-}\Delta^9\text{-THC}$, $(-)\text{-}\Delta^8\text{-THC}$, and cannabinol highly resemble the antioxidant vitamin E and have a benzopyrene moiety substituted with a phenoxy group and a hydrophobic alkyl chain. Cannabidiol contains a phenolic structure typical of many antioxidants isolated from plants. In contrast, the synthetic cannabinoid WIN 55,212-2 lacks the structural moieties that chemically define the antioxidative activity.

Cannabinoids, depending on concentration, exert at least three cellular effects via distinct mechanisms: receptor mediated, antioxidative, and cytotoxic. In immune cells at nanomolar concentrations, cannabinoids bind to CB2 and activate $\text{Gi}\alpha$ (Bayewitch et al., 1995; Slipetz et al., 1995) and mitogen-activated protein kinases (Bouaboula et al., 1996), thus may enhance cell activation as demonstrated in B-cell proliferation assays (Derocq et al., 1995). The receptor-mediated action is stereospecific and is blocked by the CB2-specific antagonist SR 144528 (Rinaldi-Carmona et al., 1998). At

submicromolar concentrations, both receptor-mediated and antioxidative mechanisms are in play. The relative importance of the two mechanisms depends on assay conditions. In low-serum or serum-free conditions, antioxidation may outweigh the CB2-mediated processes; cannabinoids, acting as antioxidants, prevent oxidative cell death and enhance cell proliferation. At concentrations $>10^{-6}$ M, the nonreceptor-mediated cytotoxic effect of cannabinoids often dominates (Schwarz et al., 1994; Zhu et al., 1998).

Cells constantly produce oxidants such as superoxide radical anion, hydrogen peroxide, and lipid peroxide (Scandalios, 1997). They rely on antioxidative enzymes such as superoxide dismutases and catalases, and small-molecule antioxidants such as vitamins A, C, E, and ubiquinol found in serum to maintain the right balance of cellular redox potential (Frei et al., 1992). Cellular oxidative stress affects cell proliferation and cell death and is involved in physiological as well as pathological events such as fertilization (Shapiro, 1991), host defense (Babior, 1978), aging (Sohal and Weindruch, 1996), tumorigenesis (Cerutti, 1985), stroke (Coyle and Puttfarcken, 1993), and AIDS (Baier-Bitterlich et al., 1996). Cannabinoids, especially the nonpsychoactive cannabinoids, may become clinically useful antioxidants in preventing and treating the oxidative stress-related diseases.

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