Original Paper

Pharmacology

Pharmacology 2010;85:328–335
DOI: 10.1159/000312686

Received: January 25, 2010
Accepted after revision: April 1, 2010
Published online: June 2, 2010

Cannabinoids Inhibit Cellular Respiration of Human Oral Cancer Cells

Donna A. Whyte[a] Suleiman Al-Hammadi[d] Ghazala Balhaj[d] Oliver M. Brown[b] Harvey S. Penefsky[c] Abdul-Kader Souid[d]

Departments of [a]Pediatrics and [b]Pharmacology, State University of New York, Upstate Medical University, Syracuse, N.Y., and [c]Public Health Research Institute, New Jersey, N.J., USA; [d]Department of Pediatrics, United Arab Emirates University, Faculty of Medicine and Health Sciences, Al Ain, United Arab Emirates

Key Words
Cannabinoids · Respiration · Mitochondria · Oral cancer

Abstract

Background and Purpose: The primary cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol (Δ^8-THC) are known to disturb the mitochondrial function and possess antitumor activities. These observations prompted us to investigate their effects on the mitochondrial O_2 consumption in human oral cancer cells (Tu183). This epithelial cell line overexpresses *bcl-2* and is highly resistant to anticancer drugs. ***Experimental Approach:*** A phosphorescence analyzer that measures the time-dependence of O_2 concentration in cellular or mitochondrial suspensions was used for this purpose. ***Key Results:*** A rapid decline in the rate of respiration was observed when Δ^9-THC or Δ^8-THC was added to the cells. The inhibition was concentration-dependent, and Δ^9-THC was the more potent of the two compounds. Anandamide (an endocannabinoid) was ineffective; suggesting the effects of Δ^9-THC and Δ^8-THC were not mediated by the cannabinoid receptors. Inhibition of O_2 consumption by cyanide confirmed the oxidations occurred in the mitochondrial respiratory chain. Δ^9-THC inhibited the respiration of isolated mitochondria from beef heart. ***Conclusions and Implications:*** These results show the cannabinoids are potent inhibitors of Tu183 cellular respiration and are toxic to this highly malignant tumor.

Introduction

The primary cannabinoids Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol (Δ^8-THC) [1] are known to have antitumor activities [2–8] and to induce apoptosis [3]. The latter cytotoxic process [9] is initiated by cell-surface receptors (e.g. Fas/APO-1) or intracellular targets (e.g., the pro-apoptotic Bcl-2 family member Bid). The resulting signals permeabilize the outer mitochondrial membrane, releasing low-molecular-weight soluble proteins (e.g., cytochrome c) from the mitochondrial intermembrane space. In the cytosol, cytochrome c binds to Apaf-1 (apoptotic protease-activating factor 1), forming oligomers (apoptosomes) that activate caspases (cysteine aspartate-directed proteases). Caspase activation leads to opening of the permeability transition pores (located at contact sites between the inner and outer mitochondrial membranes), collapse of the inner mitochondrial membrane potential ($\Delta\mu_{H+}$) and impair-

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Abdul-Kader Souid
Department of Pediatrics, United Arab Emirates University
Faculty of Medicine and Health Sciences
PO Box 17666, Al Ain (United Arab Emirates)
Tel. +971 3 713 7429, Fax +971 3 767 2022, E-Mail asouid@uaeu.ac.ae

ment of cellular respiration. These findings have been described in tumor cells treated with Δ^9-THC [10].

Tu183 cells are derived from a squamous cell carcinoma of the tonsil [11]. This epithelial cell line overexpresses *bcl-2* and is highly resistant to cytotoxic drugs [12–15].

The term cellular respiration implies delivery of O_2 and metabolic fuels to the mitochondria, oxidations of reduced fuels with passage of electrons to O_2, and synthesis of ATP. Impaired respiration thus entails an interference with any of these processes. We recently used a sensitive phosphorescence oxygen analyzer to investigate the toxic effects of doxorubicin on respiration of HL-60 (myeloid) and Jurkat (lymphoid) cells [16]. This anticancer agent inhibits mitochondrial oxygen consumption in both cell lines. We present here similar measurements for the cannabinoids on Tu183 cells.

Studies have shown that cannabinoids inhibit tumor cell proliferation, induce apoptosis and collapse $\Delta\mu_{H+}$ [2–8]. In addition, activities of cannabinoids against oral cancer cells [4] and other tumors [5] are well documented. Furthermore, the polycyclic structure of Δ^9-THC has potent effects on the inner mitochondrial membrane [17]. Therefore, we hypothesized that Δ^9-THC and Δ^8-THC impair the 'cellular bioenergetics' (oxidative phosphorylation and accompanying ATP synthesis) of Tu183 cell line. The results show for the first time that cannabinoids are potent inhibitors of respiration and ATP content in this highly malignant cell line. Moreover, light microscopy and TUNEL assay further confirmed the toxic effects of Δ^9-THC and Δ^8-THC on Tu183 cells.

Materials and Methods

Reagents

Δ^9-THC (63.7 mmol/l = 20 mg/ml 95% EtOH; MW 314.47; stored at –4°C under argon) and Δ^8-THC (127.4 mmol/l = 40 mg/ml 99% EtOH; MW 314.47; stored at –4°C under argon) were provided by the National Institute of Drug Abuse (NIDA). Laboratory standards of Δ^9-THC (1.0 mg/ml in MeOH) and Δ^8-THC (1.0 mg/ml in MeOH) were prepared by Cerilliant Corp. (Round Rock, Tex., USA) and purchased from Cambridge Isotope Laboratories, Andover, Mass., USA. Anandamide (*N*-arachidonoylethanolamine, MW 347.6) was purchased from Biomol International, LP (Plymouth Meeting, Pa., USA).

Pd (II) complex of *meso*-tetra-(4-sulfonatophenyl)-tetrabenzoporphyrin (Pd phosphor sodium salt) was purchased from Porphyrin Products (Logan, Utah, USA). Luciferin-luciferase mixture (0.2 mg luciferin and 22,000 units luciferase per vial, stored at –20°C) and ATP (2 μmol per vial, stored at –20°C) were purchased from Chrono-Log (Havertown, Pa., USA). The remaining reagents were purchased from Sigma-Aldrich.

Pd phosphor (2.0 mmol/l) was made by dissolving the powder at 2.5 mg/ml in dH_2O and stored at –20°C in small aliquots. Anandamide (25 mg) was suspended in 1.0 ml of 100% EtOH (71.9 mmol/l) and stored at –80°C under argon. NaCN (1.0 mol/l) was made in dH_2O and pH was adjusted to 7.0 with 12 N HCl. Aqueous solution of ATP (0.4 mmol/l) was made fresh in 10 mmol/l Tris-HEPES (pH 7.5); its final concentration was determined by absorbance at 259 nm, using an extinction coefficient of 15,400 $mol/l^{-1} \cdot cm^{-1}$. A working solution of ATP (8 μmol/l) was prepared immediately prior to use in 0.1 mol/l Tris-HEPES (pH 7.5), 5 mmol/l $MgCl_2$ and 0.1% fat-free bovine serum albumin. A lyophilized powder containing luciferin (0.2 mg, MW 280) and luciferase (22,000 units) was freshly dissolved in 1.25 ml phosphate-buffered saline (PBS), protected from light and placed on ice. The final concentration of luciferin (570 μmol/l) was determined by absorbance at 327 nm, using an extinction coefficient of 18,000 $mol/l^{-1} \cdot cm^{-1}$.

Cells

Tu183 cells were obtained from Professor Edward J. Shillitoe (SUNY Upstate Medical University, Syracuse, N.Y., USA). The cells were cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (Invitrogen, Carlsbad, Calif., USA) plus 10% fetal bovine serum, 1% penicillin/streptomycin and 0.2% primosin. For harvesting, the cells were incubated at 37°C in 2.5 ml of 0.05% (w/v) trypsin plus 0.53 mmol/l EDTA solution for 5 min and then collected. Each flask was carefully inspected and remaining cells were gently scraped and retrieved. The cell count was determined by light microscopy, using a hemocytometer under standard trypan blue staining conditions.

High-Performance Liquid Chromatography

Samples of the NIDA Δ^9-THC and Δ^8-THC solutions were run on high-performance liquid chromatography (HPLC), and compared to runs of the laboratory standard cerilliant Δ^9-THC and Δ^8-THC on the same system (Beckman reversed-phase HPLC with wavelength at 228 nm, 4.6 × 250 mmol/l Beckman Ultrasphere IP column at 25°C). The running solvent was acetonitrile: 0.1% phosphoric acid (75:25), isocratic at 1.5 ml/min. In all cases, the resulting chromatograms for NIDA Δ^9-THC and cerilliant Δ^9-THC were identical to one another, as were the resulting chromatograms for NIDA Δ^8-THC and cerilliant Δ^8-THC.

Cellular Respiration

$[O_2]$ in the cell suspensions was determined as a function of time, using the Pd phosphor. Samples were exposed to light flashes (10/s) from a pulsed light-emitting diode array with peak output at 625 nm (OTL630A-5-10-66-E, Opto Technology, Inc., Wheeling, Ill., USA). Emitted light was detected by a Hamamatsu photomultiplier tube after passing through a wide-band interference filter centered at 800 nm. The amplified phosphorescence decay was digitized at 1 MHz by an A/D converter (Computer Boards, Inc., Norton, Mass., USA). The phosphorescence decay of the probe was exponential, with decay rate ($1/\tau$) theoretically linear in $[O_2]$, according to $\tau^o/\tau = 1 + \tau^o\ k_q\ [O_2]$; τ, lifetime in the presence of O_2; τ^o, lifetime in the absence of O_2; and k_q, second-order O_2 quenching constant. Values of τ associated with known $[O_2]$ were determined in a series of ascorbate plus ascorbate oxidase solutions, simultaneously with electrochemical O_2 measurements on the same solutions. A plot of $1/\tau$ vs. $[O_2]$ was linear. The

quenching constant (k_q) calculated from the linear fit was 96.1 ± 1.2 $\mu m^{-1} \cdot s^{-1}$ and $1/\tau^o$ was 10,087 ± 156 s^{-1} [16].

Respiration was measured at 37°C. For each run, 1.0 ml of the cell suspension was placed in a 1.0-ml glass vial. The vial was sealed with a crimp top aluminum seal. Mixing was accomplished with the aid of a parylene-coated stirring bar. The rate of respiration (zero-order rate constant, k, in μmol/l O_2 min^{-1}) was the negative of the slope of a plot of $[O_2]$ vs. t. The addition of 10 mmol/l NaCN caused $-k = d[O_2]/dt$ to decrease almost to 0, demonstrating that the decline in $[O_2]$ with time was mainly due to mitochondrial O_2 consumption.

Mitochondrial Respiration

Measurements with mitochondria were included to demonstrate a direct effect of Δ^9-THC on the mitochondrial respiratory chain [17]. Mitochondria were prepared from beef heart as described [18]. Mitochondria were suspended in 1.0 ml of 10 mmol/l Tris-Cl (pH 8.2), 250 mmol/l sucrose, 2.0 μmol/l Pd phosphor and 0.5% albumin. The mixture was transferred to a 1.0-ml glass vial and placed in the instrument for O_2 measurement. O_2 consumption was initiated by the addition of 50 mmol/l succinate. Where shown, other additions were 2 μl EtOH (control), 120 μmol/l Δ^9-THC, 10 mmol/l NaCN and glucose (1.0 mmol/l) plus glucose oxidase (7.0 units). Glucose oxidase catalyzes the reaction: β-D-glucose + O_2 → D-glucono-1,5-lactone + H_2O_2.

Cellular ATP

ATP was measured in Tu183 cell acid extracts that were prepared by adding 400 μl of 10% perchloric acid to pellets containing 2.4 × 10^5 cells. The mixtures were sonicated on ice for 30 s and the supernatants were neutralized with 400 μl of 2.0 mol/l KOH. The samples were incubated on ice for 15 min and the precipitated $KClO_4$ was removed by centrifugation.

The luciferin-luciferase bioluminescence system was used to determine cellular ATP. Luminescence was measured at 37°C using a luminometer (Chrono-Log Corporation, Havertown, Pa., USA) connected to Chrono-log AGGRO/LINK™ interface and analyzed as described [16]. The reaction (final volume, 0.4 ml) contained 0.1 mol/l Tris-HEPES (pH 7.6), 5 mmol/l $MgCl_2$, 0.1% albumin and ATP (5–40 pmol) or cellular acid extracts (25 μl). The reaction was started by injecting 10 μl of luciferin/luciferase mixture (5 nmol luciferin plus 176 units luciferase). The luminometer was calibrated with known amounts of ATP (5–40 pmol). The intensity was measured every 0.5 s out to 600 s. Plots of luminescence intensity I vs. t were obtained and fitted to: $I = a + b\, e^{-ct}$ ($r > 0.9556$). Cellular ATP content was calculated as described [16].

TUNEL Assay

DNA fragmentation was visualized using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) technique (In situ Cell Death Detection Kit, Fluorescein; Roche Applied Science, Mannheim, Germany). Cells were plated at 2.0 × 10^5 per well in chamber slides overnight. On the day of treatment, media with EtOH (control) or Δ^9-THC (120 μmol/l) were added and the cells were incubated at 37°C for 60 min. The slides were air dried, fixed in 4% (v/v) paraformaldehyde (in PBS, pH 7.4) and rinsed in PBS. The slides were then incubated on ice in the permeabilisation solution (0.1% (v/v) Triton X-100 in 0.1% (w/v) sodium citrate) for 2 min and rinsed twice in PBS. 50 μl of the TUNEL reaction mixture was added. The slides were covered with coverslips, incubated at 37°C in the dark for 1 h, and rinsed 3 times with PBS. Vectashield (Vector Laboratories, Inc., Burlingame, Calif., USA) and coverslips were placed before viewing under a Nikon fluorescence microscope, with an exciter filter at 465–495 nm and a barrier filter at 515–555 nm.

Results

Cannabinoids Inhibited Tu183 Cellular Respiration

Cells (0.5 × 10^6/ml) were suspended in media containing 2 μmol/l Pd phosphor and 0.5% albumin with and without 200 μmol/l Δ^9-THC or Δ^8-THC. The plots of $[O_2]$ as function of time are shown in figure 1a. The rate of cellular mitochondrial O_2 consumption (k, in μmol/l O_2/min) for untreated cells was 2.6 ($r^2 = 0.991$), for cells treated with MeOH 2.8 ($r^2 = 0.988$; added as a control for the cannabinoids' vehicle), for cells treated with Δ^9-THC 0.5 ($r^2 = 0.903$, 81% inhibition), and for cells treated with Δ^8-THC 0.9 ($r^2 = 0.973$, 65% inhibition). Cyanide (10 mmol/l) gave almost complete inhibition (≥84%); confirming the decline in $[O_2]$ was mainly due to mitochondrial O_2 consumption. The k values for media (without cells) plus Δ^9-THC or Δ^8-THC were very similar to the instrument drift (0.03 μmol/l O_2 min^{-1}). Thus, potential interactions between cannabinoids and O_2 or Pd phosphor are negligible.

Dependence of the inhibition on Δ^9-THC dosing is shown in figure 1b. O_2 consumption was measured in Tu183 cells (0.5 × 10^6/ml) in the presence of 0–100 μmol/l Δ^9-THC. The value of k (μmol/l O_2 min^{-1}) for untreated cells was 1.7, for cells treated with 10 μmol/l Δ^9-THC 1.9, for cells treated with 20 μmol/l Δ^9-THC 1.7, for cells treated with 50 μmol/l Δ^9-THC 1.0 (41% inhibition), and for cells treated with 100 μmol/l Δ^9-THC 0.8 (53% inhibition). The same experiment was repeated with Δ^8-THC (not shown). The value of k for untreated cells was 1.9, for cells treated with 10 μmol/l Δ^8-THC 2.0, for cells treated with 20 μmol/l Δ^8-THC 2.0, for cells treated with 50 μmol/l Δ^8-THC 1.8, and for cells treated with 100 μmol/l Δ^8-THC 1.3 (32% inhibition). In other experiments, the inhibition with 120 μmol/l Δ^9-THC was 48%, with 150 μmol/l Δ^9-THC 76%, and with 100 μmol/l Δ^8-THC 39%. Thus, the cannabinoids produced dose-dependent inhibition of Tu183 cellular respiration.

In another experiment, cells (0.5 × 10^6/ml) were treated at 37°C with 4 μl MeOH or 25 μmol/l Δ^8-THC (both added to 5-ml cell suspensions at minute zero). The cells were then harvested at 0 and 108 min, respectively for O_2 measurement. The value of k (μmol/l O_2 min^{-1}) for un-

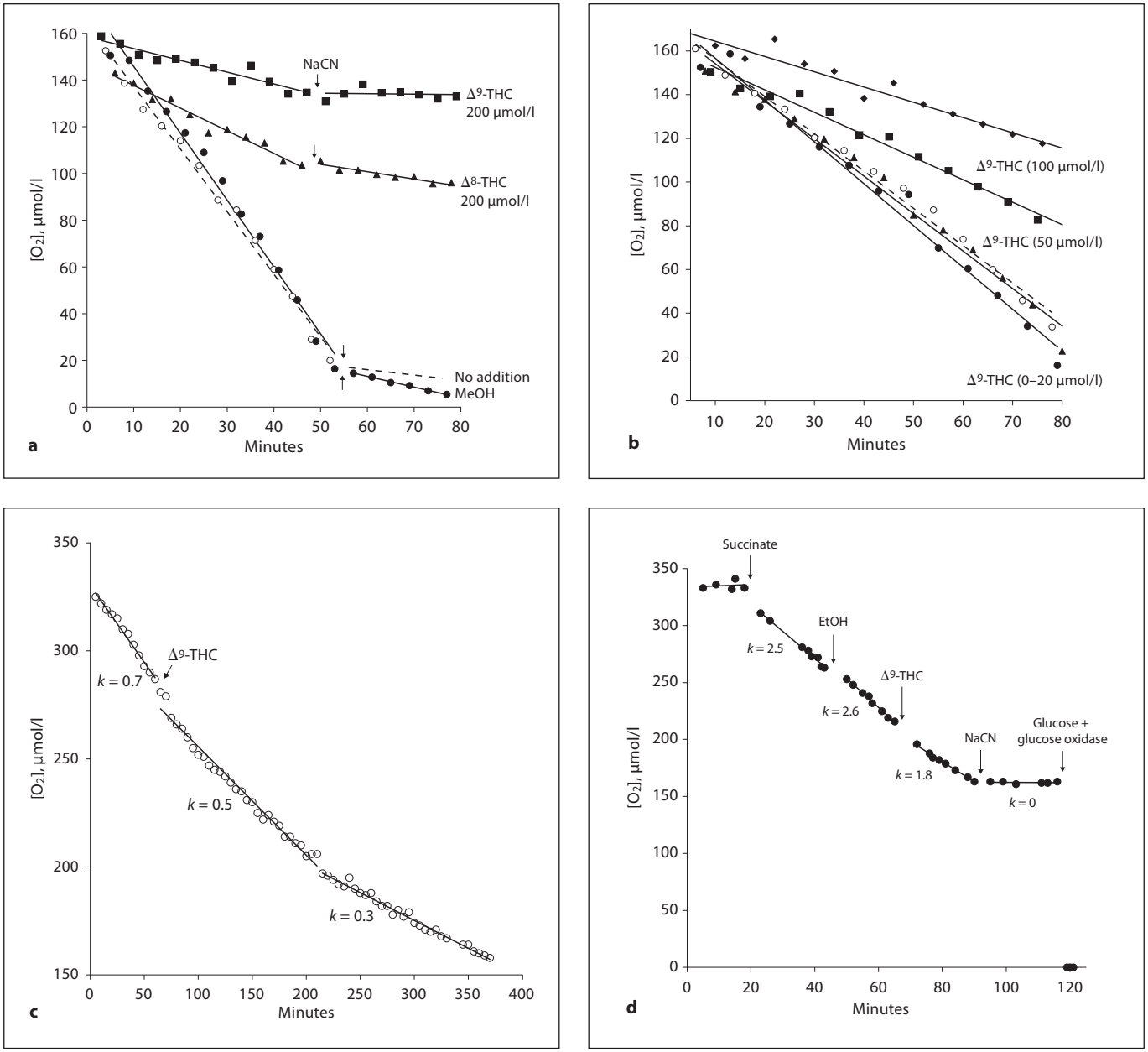

Fig. 1. Cannabinoids inhibit Tu183 cellular respiration. **a** TU183 cells (1.0×10^6/ml) were suspended in media containing 2 μmol/l Pd phosphor and 0.5% albumin with no addition (open circles and dashed line, $r^2 > 0.991$) or with the addition of MeOH (closed circles and solid line, $r^2 > 0.988$), 200 μmol/l Δ^9-THC (squares and solid line, $r^2 > 0.903$), or 200 μmol/l Δ^8-THC (triangles and solid line, $r^2 > 0.973$). Rates of respiration (k, μmol/l O_2 min^{-1}) were set as the negative of the slopes. The lines are linear fits. The addition of 10 mmol/l NaCN (arrows) is shown. **b** Respiration of TU183 cells (0.5×10^6/ml) were measured in the presence of 0–100 μmol/l Δ^9-THC. Open circles and dotted lines ($r^2 > 0.987$), untreated; closed circles and solid lines ($r^2 > 0.980$), 10 μmol/l; triangles and solid lines ($r^2 > 0.988$), 20 μmol/l; squares and solid lines ($r^2 > 0.980$), 50 μmol/l; diamonds and solid line ($r^2 > 0.960$), 100 μmol/l. **c** Respiration of TU183 cells (0.8×10^6 cells/ml) with injection of 120 μmol/l Δ^9-THC. Best-fit curves ($r > 0.977$) and values of k (μmol/l O_2 min^{-1}) are shown. **d** Δ^9-THC inhibited O_2 consumption by isolated mitochondria (12.5 μg) from beef heart. O_2 consumption was initiated with 50 mmol/l succinate. Other additions were 2 μl EtOH, 120 μmol/l Δ^9-THC, 10 mmol/l NaCN, and glucose (1.0 mmol/l) + glucose oxidase (7.0 units). Best-fit curves ($r > 0.994$) and values of k (μmol/l O_2 min^{-1}) are shown.

treated cells was 1.5 and for Δ^8-THC-treated cells 1.6. Thus, Δ^8-THC at $\leq$25 μmol/l was ineffective, even with 2 h exposure. The same experiment was then repeated with 100 μmol/l Δ^8-THC. Cells (2.0×10^6/ml) were treated at 37°C without or with 100 μmol/l Δ^8-THC. The cells were harvested at minutes 260 and 290, respectively, for O_2 measurement. The value of k (μmol/l O_2 min^{-1}) for MeOH-treated cells was 9.5 and for Δ^8-THC-treated cells 1.9 (80% inhibition). Thus, a prolonged exposure to 100 μmol/l Δ^8-THC augmented the inhibition. The experiment was also repeated with 40 μmol/l Δ^9-THC. Cells (0.5×10^6/ml) were treated at 37°C with 3 μl EtOH or 40 μmol/l Δ^9-THC (both added to 5-ml cell suspensions at zero minutes). The cells were harvested at minutes 124 and 227, respectively, for O_2 measurement. The value of k for EtOH-treated cells was 1.8 μmol/l O_2 min^{-1} and for Δ^9-THC-treated cells 1.7 μmol/l O_2 min^{-1}. Thus, Δ^9-THC at $\leq$40 μmol/l was ineffective, even with a long exposure.

To precisely determine the onset of respiratory inhibition, Δ^9-THC (120 μmol/l) was injected into the cell suspension during O_2 measurement. The value of k (μmol/l O_2 min^{-1}) before any injection was 1.1 ($4 \text{ min} < t < 30$ min), after injecting 2 μl EtOH 1.3 ($35 \text{ min} < t < 60$ min), after injecting 120 μmol/l Δ^9-THC 1.0 ($69 \text{ min} < t < 96$ min; 23% inhibition) and 0.6 ($99 \text{ min} < t < 126$ min; 54% inhibition), and after injecting 10 mmol/l NaCN 0.4 ($130 \text{ min} < t < 155$ min; 69% inhibition). The remaining O_2 was rapidly consumed when glucose oxidase was injected. The same experiment was repeated (fig. 1c). The value of k before the injection was 0.7 ($0 \text{ min} < t < 60$ min) and after injecting 120 μmol/l Δ^9-THC 0.5 ($65 \text{ min} < t < 215$ min; 29% inhibition) and 0.3 ($215 \text{ min} < t < 370$ min; 57% inhibition). Thus, Δ^9-THC caused immediate inhibition of respiration followed by further inhibition after ~150 min. Rapid inhibition was also observed with injecting 174 μmol/l Δ^8-THC (not shown).

Anandamide (120 μmol/l), an endocannabinoid, did not inhibit cellular mitochondrial O_2 consumption, suggesting the effect on respiration was not mediated by cannabinoid receptors.

ATP content was measured in Tu183 cells following incubation at 37°C for 60 min with 12 μl EtOH or 150 μmol/l Δ^9-THC (both added to 5-ml cell suspensions). For EtOH-treated cells, the average ± SD of ATP level (n = 3) was 1.28 ± 0.09 nmol for 2.4×10^5 cells. The presence of Δ^9-THC led to ~64% decrease in the ATP level, 0.46 ± 0.04 nmol for 2.4×10^5 cells. Addition of 10 mmol/l NaCN to the cells resulted in 22% decrease in the ATP level at 1 h, suggesting Δ^9-THC treatment caused cellular ATP leak.

Figure 1d shows the effect of Δ^9-THC on the respiration of isolated mitochondria from beef heart. In the presence of 120 μmol/l Δ^9-THC, the value of k deceased from 2.6 to 1.8 μmol/l O_2 min^{-1} (30% inhibition). Complete inhibition of the respiration was observed with 10 mmol/l NaCN; O_2 depletion was then observed with glucose plus glucose oxidase.

Figure 2a, b shows the morphologic effects of Δ^9-THC treatment (120 μmol/l for 60 min) on Tu183 cells. Loss of cell-to-cell contact, nuclear condensation, membrane blebbing, cytoplasmic condensation and cytoplasmic vacuolization are evident. Some of these features were present as early as 25 min into the drug treatment. The ability of Δ^9-THC to induce DNA fragmentation was then investigated by the TUNEL assay (fig. 2c, d). The cells were incubated at 37°C with EtOH (fig. 2c) or 120 μmol/l Δ^9-THC (fig. 2d) for 60 min. Positive cells were noted with Δ^9-THC (fig. 2d). No positive cells were seen when the cells were treated with 120 μmol/l Δ^9-THC for ≤30 min (data not shown). In the viability test, the percentage (mean ± SD, n = 12) of trypan blue-positive cells for EtOH-treated cells was 1.8 ± 1.0 and for Δ^9-THC-treated cells (120 μmol/l for 60 min) 6.5 ± 4.3 ($p < 0.003$).

The effects of cannabinoids were then compared with that of other anticancer drugs. Tu183 cells are known to be resistant to chemotherapeutic agents. Therefore, a longer exposure to the drugs was used. The cells were treated at 37°C with 2 μl dimethyl sulfoxide (control) or 10 μmol/l camptothecin for 5 h. The rates of respiration for the two conditions were the same (1.1 μmol/l O_2 min^{-1}). In another experiment, the cells were treated at 37°C with 25 μmol/l camptothecin, carboplatin, oxaliplatin, cisplatin or doxorubicin for 20 h. Inhibition of respiration (55%) was observed only in the cisplatin-treated cells (fig. 3).

Discussion

Oral squamous cell carcinoma is the 6th most common malignancy. This cancer is responsible for the death of over 8,000 patients per year in the US [20]. The tumor remains one of the hardest to treat, not only because of its poor response to therapy, but also due to physical complications posed by the surgery [21]. Moreover, oral cancer cells (e.g., Tu183 cells) are known to adapt genetic changes that block cell death. Examples of such adaptations include mutations of *p53* and upregulation of *bcl*-2 expression [12].

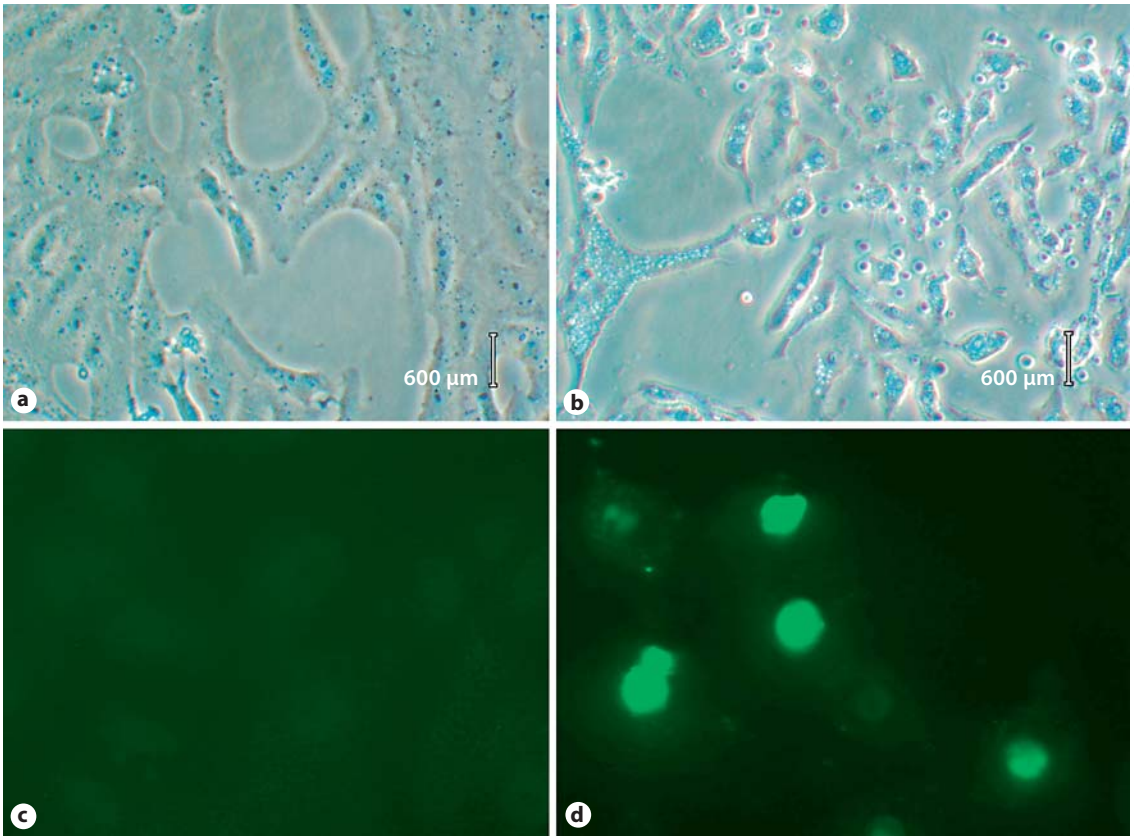

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Fig. 2. Bright-field microscopy of Tu183 cells. ×20. **a**, **b** Cells at 85% confluence in 5-ml media were treated at 37°C with 10 µl EtOH (**a**) or 120 µmol/l Δ^9-THC (**b**) for 60 min. TUNEL assay. ×100. **c**, **d** Cells were grown in chamber slides (500 µl/well) overnight, and then treated at 37°C with 1.0 µl EtOH (**c**) or 120 µmol/l Δ^9-THC (**d**) for 60 min.

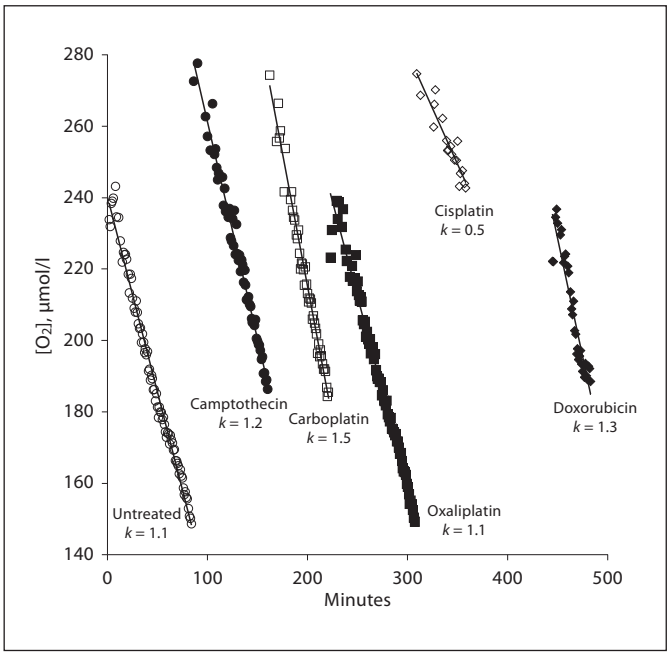

Fig. 3. Effects of anticancer drugs on Tu183 cellular respiration. Cells were treated at 37°C with 25 µmol/l camptothecin, carboplatin, oxaliplatin, cisplatin or doxorubicin for 20 h. The cells were then harvested, suspended in media plus 2.0 µmol/l Pd phosphor plus 0.5% albumin, and analyzed for O_2 consumption. Best-fit curves ($r > 0.939$) and values of k (µmol/l O_2 min^{-1}) are shown.

The Tu183 cells are derived from a squamous cell carcinoma of the tonsil [11]. This cell line, as shown in figure 3, is highly resistant to chemotherapeutic drugs [13]. We report here on the inhibitory effects of Δ^9-THC and Δ^8-THC on Tu183 cellular respiration (fig. 1). As early as 1975, Δ^9-THC was shown to reduce growth of lung tumors in mice [22]. Studies have also shown that cannabinoids inhibit tumor cell proliferation, induce apoptosis and collapse $\Delta\mu_{H+}$ [2–8, 23]. These effects are functions of drug concentration and exposure time. For example, loss of $\Delta\mu_{H+}$ was observed in cultured pulmonary cells exposed to 40 μmol/l Δ^9-THC for 1 h [10]. Moreover, in mice, in vivo treatment with Δ^9-THC produced lymphocyte atrophy (apoptosis) at about 6 h [3], presumably due to release of the pro-apoptotic immune modulator interleukin-1 and block of the anti-apoptotic protein Bcl-2 [8]. In addition, activities of cannabinoids against oral cancer cells [4] and other tumors [5] have been reported.

Our results show that cannabinoids (especially Δ^9-THC) are potent inhibitors of Tu183 cell respiration (fig. 1). Furthermore, the effect of Δ^9-THC is immediate (fig. 1c) and more potent than that observed with commonly used anticancer drugs (fig. 3). On the other hand, anandamide (an endocannabinoid) is ineffective, suggesting that the effects of Δ^9-THC and Δ^8-THC are not mediated via the cannabinoid receptors.

The mechanism of cannabinoid-mediated impairment of cellular respiration (inhibition of mitochondrial O_2 consumption and accompanying ATP synthesis) remains unknown. Δ^9-THC is known to interfere with components of the mitochondrial respiratory chain [17]. In addition to a direct inhibition of oxidative phosphorylation [17], Δ^9-THC-induced proteases may impair other processes in ATP synthesis or hydrolysis [19]. Attempts to block the effect of Δ^9-THC on respiration by using the pan-caspase inhibitor N-acetyl-asp-glu-val-asp-7-amino-4-trifluoromethyl coumarin were complicated by the fact that dimethyl sulfoxide (the vehicle) itself increased respiration in Tu183 cells. Since the inhibition of respiration is immediate (fig. 1c), it is unlikely that caspase activation (a timed process) is involved in the beginning. However, caspases could be activated later, as a consequence of decreased respiration or drug-induced apoptosis. These possibilities need further investigation.

The fact that Δ^9-THC inhibits the respiration of isolated mitochondria from beef heart (fig. 1d) suggests a direct toxic interaction of the compound with mitochondria. It has been argued [17] that the polycyclic structure of Δ^9-THC can have strong effects on membrane-dependent processes such as those of the inner mitochondrial membrane. Future studies are necessary to determine whether the cannabinoids can be used to treat patients with oral cancer.

Acknowledgements

This work is supported by a fund from Paige's Butterfly Run. The contribution of Professor Jerry Goodisman is greatly appreciated.

References

1 Gaoni Y, Mechoulam R: Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964;86:1646–1647.

2 Neu R, Powers H, King S, Gardner LI: RΔ^8- and Δ^9-tetrahydrocannabinol: effects on cultured human leucocytes. J Clin Pharmacol New Drugs 1970;10:228–230.

3 McKallip RJ, Lombard C, Martin BR, Nagarkatti M, Nagarkatti PS: MΔ^9-Tetrahydrocannabinol-induced apoptosis in the thymus and spleen as a mechanism of immunosuppression in vitro and in vivo. J Pharmacol Exp Ther 2002;302:451–465.

4 Baek SH, Kim YO, Kwag JS, Choi KE, Jung WY, Han DS: Boron trifluoride etherate on silica-A modified Lewis acid reagents (VII): antitumor activity of cannabigerol against human oral epitheloid carcinoma cells. Arch Pharm Res 1998;21:353–356.

5 Sarfaraz S, Adhami VM, Syed DN, Afaq F, Mukhtar H: Cannabinoids for cancer treatment: progress and promise. Cancer Res 2008;68:339–342.

6 Bifulco M, Laezza C, Pisanti S, Gazzerro P: Cannbinoids and cancer: pros and cons of an antitumor strategy. Br J Pharmacol 2006;148:123–135.

7 Kogan NM: Cannbinoids and cancer. Mini Rev Med Chem 2005;5:941–952.

8 Zhu W, Friedman H, Klein TW: Δ^9-Tetrahydrocannabinol induces apoptosis in macrophages and lymphocytes: involvement of Bcl-2 and caspase-1. J Pharmacol Experimental Therapeutics 1998;286:1103–1109.

9 Batandier C, Leverve X, Fontainer E: Opening of the mitochondrial permeability transition pore induces reactive oxygen species production at the level of the respiratory chain complex I. J Biol Chem 2004;279:17197–17204.

10 Sarafian TA, Kouyoumjian S, Khoshaghideh F, Tashkin DP, Roth MD: Δ^9-Tetrahydrocannabinol disrupts mitochondrial function and cell energetics. Am J Physiol Lung Cell Mol Physiol 2003;284:L298–L306.

11 Sacks PG, Parnes SM, Gallick GE, Mansouri Z, Lichtner R, Satya-Prakash KL, Pathak S, Parsons DF: Establishment and characterization of two new squamous cell carcinoma cell lines derived from tumors of the head and neck. Cancer Res 1988;15:2858–2866.

12 Gibson S, Shillitoe E: Analysis of apoptosis-associated genes and pathways in oral cancer cells. J Oral Pathol Med 2006;35:146–154.

13 Gibson S, Pellenz C, Hutchinson RE, Davey FR, Shillitoe EJ: Induction of apoptosis in oral cancer cells by an anti-bcl-2 ribozyme delivered by an adenovirus vector. Clin Cancer Res 2000;6:213–222.

14 Yao L, Iwai M, Furata I: Correlations of bcl-2 and p53 expression with the clinicopathological features in tongue squamous cell carcinomas. Oral Oncol 1999;35:56–62.
15 Whyte DA, Broton CE, Shillitoe EJ: The unexplained survival of cells in oral cancer: what is the role of p53? J Oral Pathol Med 2002;31:125–133.
16 Tao Z, Withers HG, Penefsky HS, Goodisman J, Souid AK: Inhibition of cellular respiration by doxorubicin. Chem Res Toxicol 2006;19:1051–1058.
17 Mahoney JM, Harris RA: Effect of Δ^9-tetrahydrocannabinol on mitochondrial processes. Biochem Pharmacol 1972;21:1217–1226.
18 Beyer RE: Preparation, properties, and conditions for assay of phosphorylating electron transport particles (ETPH) and its variations. Methods Enzymol 1967;10:186–194.
19 Toro-Goyco E, Rodriguez MB, Preston AM: On the action of Δ^9-tetrahydrocannabinol as an inhibitor of sodium- and potassium-dependent adenosine triphosphatase. Mol Pharmacol 1978;14:130–137.
20 World Health Organization: The World Oral Health Report 2003. Geneva, World Health Organization, 2003, pp 6–7.
21 Centers for Disease Control and Prevention: Preventing and controlling oral and pharyngeal cancer: recommendations from a national strategic planning conference. MMWR 1998;47:1–12.
22 Munson AE, Harris LS, Friedman MA, Dewey WL, Carchman RA: Antineoplastic activity of cannabinoids. J Natl Cancer Inst 1975; 55:597–602.
23 Casanova ML, Blazquez C, Martinez-Palacio J, Villanueva C, Fernandez-Acenero MJ, Huffman JW, Jorcano JL, Guzman M: Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. J Clin Invest 2003;111:43–50.