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Cannabidiol (CBD) Prevents Palmitic Acid-Induced Drop in Mitochondrial Membrane Potential

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Abstract

Exposure of macrophages and microglia cells to the saturated palmitic acid (PA) leads to reduction in the mitochondrial membrane potential ($\Delta\Psi_m$), shrinkage of the cells and apoptosis. Here we show that the Cannabis component Cannabidiol (CBD) rescues both macrophages and microglia cells from the detrimental effects of PA. CBD prevents the shrinkage in cell size and the reduction in $\Delta\Psi_m$ caused by PA. The protective effect of CBD on the macrophage mitochondria is important for sustaining the macrophage population even under the immunosuppressed conditions caused by this drug. To a similar extent, the antagonistic effect of CBD on PA-mediated microglia cytotoxicity is important for its role in neuroprotection.

Keywords

CBD, Mitochondrial Membrane Potential, Macrophages, Microglia, Palmitic Acid

1. Introduction

Cannabidiol (CBD) is known to exert strong anti-inflammatory and anti-nociceptive properties [1]-[6], besides its well-known anti-microbial activities [7]. In addition, CBD has been shown to be neuroprotective and possess anti-oxidative activities [8]. These promiscuous properties have made CBD a broad-spectrum attractive drug for the treatment of a wide range of diseases such as autoimmune diseases, rheumatoid arthritis, multiple sclerosis and diabetes, and for the alleviation of cancer-associated and other disease-associated pain [6] [9]. In addition,

CBD has been shown to exert anti-epileptic, anxiolytic and anti-psychotic activities [8].

Δ^9-tetrahydrocannabinol (THC), CBD and their derivatives have previously been shown to modulate several macrophage functions such as inhibition of phagocytosis and chemotaxis as well as repression of TNF*α*, reactive oxygen species (ROS) and nitric oxide (NO·) production [4] [6] [10] [11] [12] [13]. CBD also alters microglial gene expression in response to lipopolysaccharides, with a prominent elevation of Nrf2-regulated genes involved in oxidant defense and redox signaling [14].

Palmitic acid (PA) leads to apoptosis of macrophages in a CD36-dependent manner [15] [16]. It causes apoptosis through generation of ROS by the NADPH oxidase subunit NOX2 [15]. PA also activates microglia cells, leading to their increased secretion of pro-inflammatory cytokines, ROS and NO· production, which ultimately trigger bystanding neuronal death [17].

Here we show that CBD rescues macrophages and microglia cells from the cytotoxic effects of PA. Of particular interest is the antagonistic effect of CBD on the PA-mediated drop in mitochondrial membrane potential.

2. Material and Methods

2.1. Reagents

Purified Cannabidiol (CBD; THC Pharm. GmbH, Frankfurt, Germany) was dissolved in ethanol:Cremophor:saline at a 1:1:18 ratio. Palmitic acid (PA; Sigma) was dissolved in ethanol, and then diluted in DMEM containing 1% heat-inactivated fetal calf serum (FCS) and 1% bovine serum albumin. Red MitoTracker CM-H_2XRos (Molecular Probes, Life Technologies) was dissolved in DMSO.

2.2. Cell Culture

RAW 264.7 macrophage cell line and BV-2 microglial cell lines were grown in DMEM supplemented with 10% heat-inactivated FCS, and incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

2.3. Treatments

RAW 264.7 or BV-2 cells were incubated with 75 - 150 μM PA in the absence or presence of 1 - 5 μg/ml CBD for 24 hrs. 30 minutes before the end of incubation, the cells were exposed to 10 μM Red MitoTracker. Immediately thereafter, the cultures were inspected by confocal microscopy (Nikon A1 HD25). The relative red intensity was calculated by ImageJ measurements divided by number of cells. Control cells received the respective vehicles.

2.4. Statistics

The results are presented as average ± standard error. p-values were calculated from two-tail Student's t-tests. A p-value equal or below 0.05 was considered sta-

tistically significant.

3. Results

3.1. Cannabidiol (CBD) Prevents Palmitic Acid (PA)-Induced Drop in Mitochondrial Membrane Potential in Macrophages

Palmitic acid (PA) has previously been shown to induce apoptosis of macrophages [15] [16]. Here we show that a 24 hrs incubation with palmitic acid leads to strong reduction in mitochondrial membrane potential (**Figure 1**; compare D-F with A-C) with concomitant shrinkage and rounding up of cells with occasional appearance of blebbing cells indicative for apoptosis (**Figure 2**; compare D-F with A-C). ImageJ analysis of red fluorescence intensity/cell shows an 80% ± 4% reduction in MitoTracker staining of PA-treated macrophages (**Figure 3(A)**; $p = 0.02$). However, when the macrophages were simultaneously treated with CBD, the PA-induced drop in $\Delta\Psi_m$ was significantly prevented (**Figure 1**; compare G-I with D-E; and **Figure 3(A)** with a $p = 0.002$). ImageJ analysis

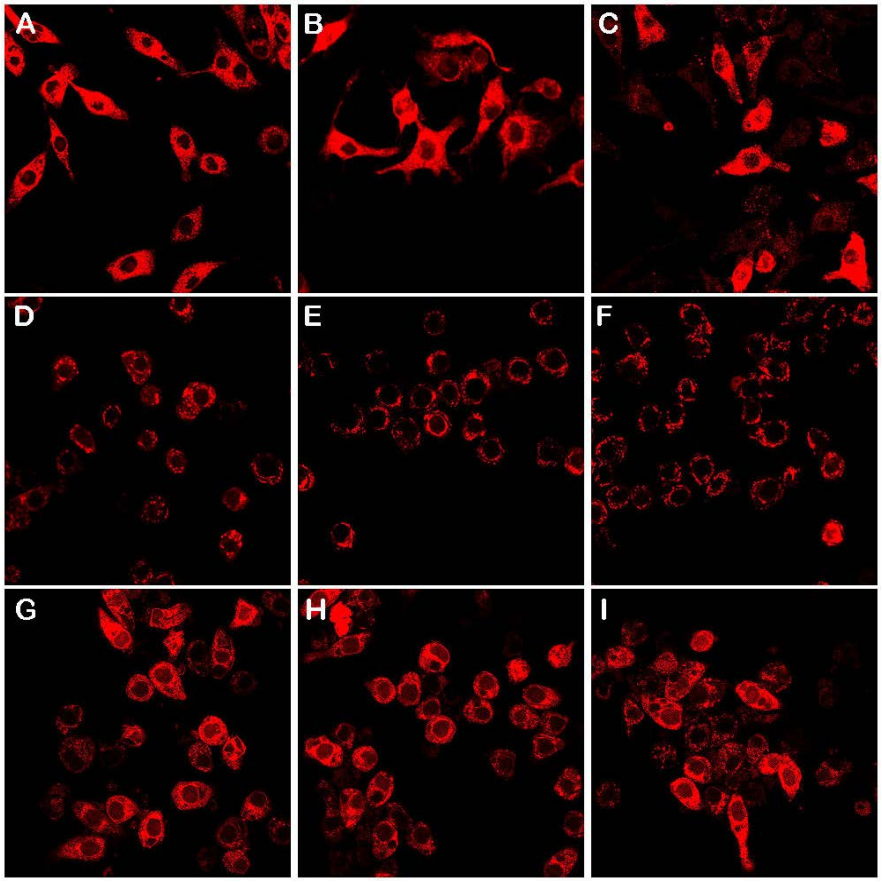

Figure 1. CBD protects RAW 264.7 macrophages from PA-induced drop in mitochondrial membrane potential. RAW 264.7 macrophages were incubated in the absence or presence of 75 μM PA alone or together with 5 μg/ml CBD for 24 hrs. Red MitoTracker was added to a final concentration of 10 μM 30 min before the end of incubation. (A) - (C) Macrophages incubated with vehicle only; (D) - (F) Macrophages incubated with 75 μM PA; (G) - (I) Macrophages incubated with 75 μM PA and 5 μg/ml CBD.

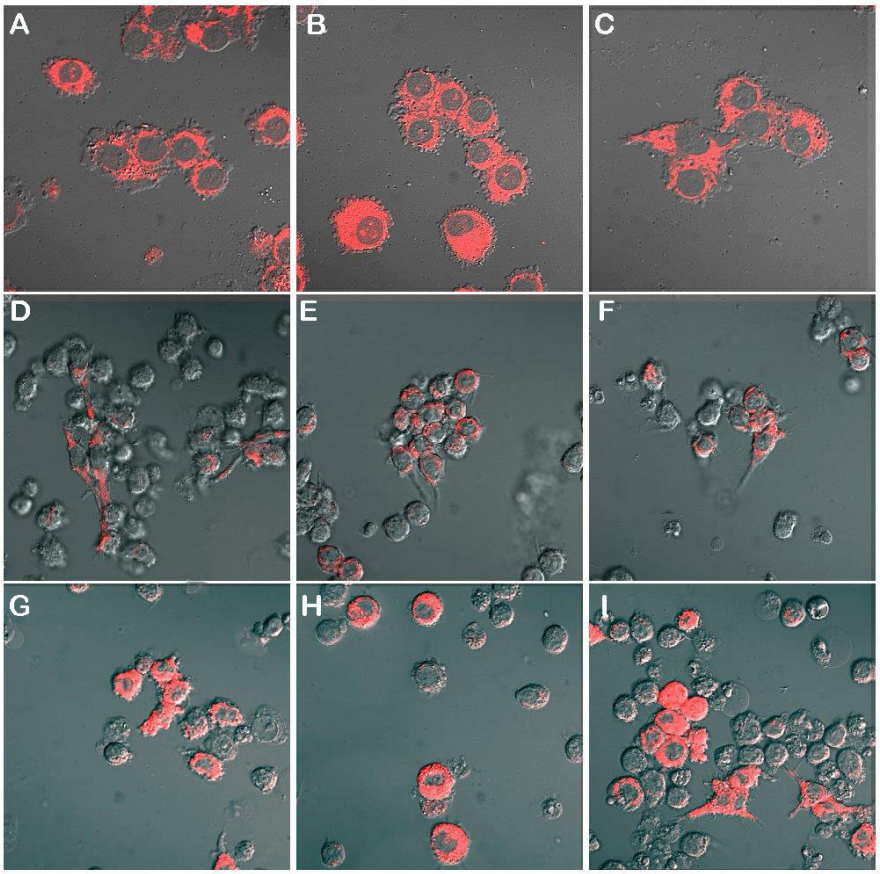

Figure 2. CBD protects RAW 264.7 macrophages from PA-mediated cytotoxicity. RAW 264.7 macrophages were incubated in the absence or presence of 150 μM PA alone or together with 5 μg/ml CBD for 24 hrs. Red MitoTracker was added to a final concentration of 10 μM 30 min before the end of incubation. (A) - (C) Macrophages incubated with vehicle only; (D) - (F) Macrophages incubated with 150 μM PA; (G) - (I) Macrophages incubated with 150 μM PA and 5 μg/ml CBD.

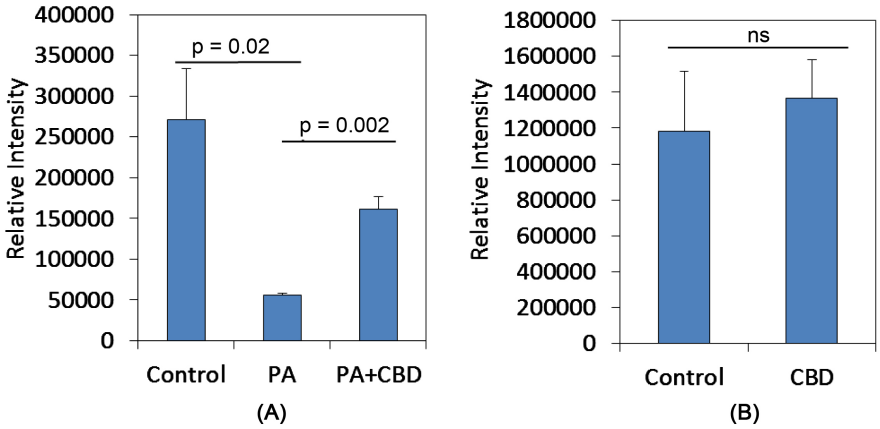

Figure 3. The relative red fluorescence intensity of macrophages treated with PA and/or CBD. (A) The relative red fluorescence of Red MitoTracker staining of macrophages treated with vehicle (Control), 75 μM PA alone or 75 μM PA together with 5 μg/ml CBD for 24 hrs; (B) The relative red fluorescence of Red MitoTracker staining of macrophages treated with vehicle (Control) or 5 μg/ml CBD for 24 hrs.

showed that macrophages co-treated with CBD and PA had only a 40% ± 5% reduction in MitoTracker staining in comparison to control macrophages (**Figure 1**; compare G-H with A-C). Compared to PA-treated macrophages, there was a 3-fold increase in MitoTracker staining in the presence of CBD. CBD also prevented the rounding up of macrophages (**Figure 1** and **Figure 2**), suggesting for a pro-survival effect. The CBD protection was more profound when the macrophages were treated with 75 μM PA (**Figure 1**), than when treated with 150 μM PA (**Figure 2**). Macrophages treated with CBD alone showed similar MitoTracker staining as control cells (**Figure 3(B)** and **Figure 4**).

3.2. CBD Prevents PA-Mediated Cytotoxicity of Microglia Cells

Palmitic acid has previously been shown to reduce the viability of microglia cells [18]. This is also demonstrated here by exposing BV-2 microglia cells to 75 μM PA for 24 hrs resulting in a 55% ± 5% reduction in cell viability (**Figure 5** compare D-F to A-C; and **Figure 6**). Simultaneous exposure of microglia cells to both 75 μM PA and 5 μg/ml CBD prevented the cytotoxic effect of PA (**Figure 5** compare G-I with D-F; and **Figure 6**), suggesting for a protective role for CBD.

4. Discussion

Cannabidiol (CBD) has repeatedly been shown to exert immunosuppressive effects *in vivo* [1] [2] [3] [4]. *In vitro*, CBD has been shown to suppress several macrophage functions such as phagocytosis, and the production of TNF*α*, ROS and NO· in response to stimulus [4] [6] [10] [11] [12] [13]. Here we have shown that CBD antagonizes the cytotoxic effects of PA on both macrophages and microglia cells. CBD prevented the drop in mitochondrial membrane potential

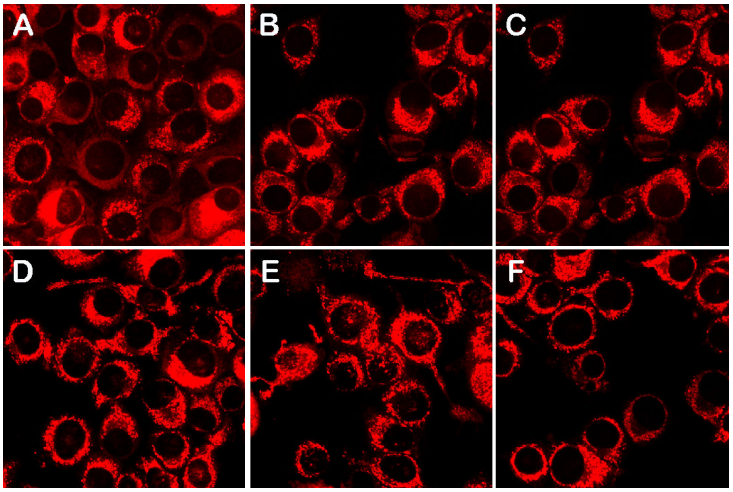

Figure 4. Red Mitotracker staining of control and CBD-treated macrophages. RAW 264.7 macrophages were incubated in the absence or presence of 5 μg/ml CBD for 24 hrs. Red MitoTracker was added to a final concentration of 10 μM 30 min before the end of incubation. (A) - (C) Macrophages incubated with vehicle only; (D) - (F) Macrophages incubated with 5 μg/ml CBD.

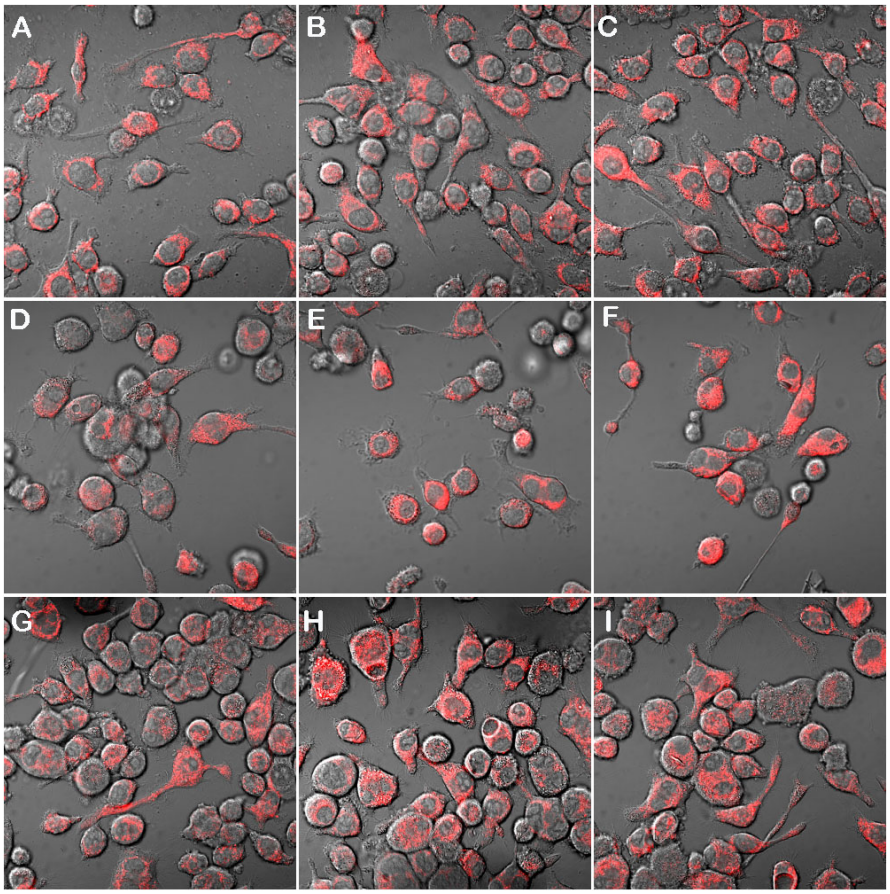

Figure 5. CBD antagonizes the cytotoxic effects of PA on microglia cells. BV-2 microglia cells were incubated in the absence or presence of 75 μM PA alone or together with 5 μg/ml CBD for 24 hrs. Red MitoTracker was added to a final concentration of 10 μM 30 min before the end of incubation. (A) - (C) BV-2 microglia cells incubated with vehicle only; (D) - (F) BV-2 microglia cells incubated with 75 μM PA; (G) - (I) BV-2 microglia cells incubated with 75 μM PA and 5 μg/ml CBD.

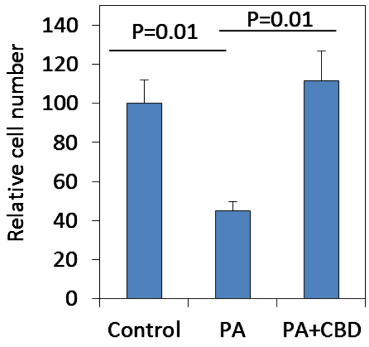

Figure 6. The relative number of microglia cells treated with PA alone or together with CBD. BV-2 microglia cells were incubated in the absence or presence of 75 μM PA alone or together with 5 μg/ml CBD for 24 hrs. The relative cell number is presented in comparison to vehicle-treated cells (Control).

caused by PA. A recent study has observed a similar protection of hepatocytes from PA-induced apoptosis by CBD [19]. These authors observed that PA re-

duced the mitochondrial membrane potential and increased the mitochondrial reactive oxygen species production in hepatocytes, both effects antagonized by CBD [19]. Also the neuroprotective activity of CBD has been related to its effects on mitochondria [20], where CBD under pathophysiological conditions prevented apoptosis via restoration of Ca^{2+} homeostasis. CBD interacts with Ca^{2+} TRP channels [6], which might account for this effect. The pro-survival effect of CBD on macrophages observed in the present study is intriguing in light of its ability to suppress various macrophage functions. This characteristic of CBD is important for sustaining the macrophage population even under immunosuppressed conditions. Similarly, the pro-survival effect of CBD on microglia is important for neuroprotection. In both macrophages and microglia cells, there is a correlation between the documented reduction in ROS and NO· production caused by CBD and the preservation of mitochondrial function described in the present study. It is thus likely that the anti-oxidant properties of CBD contribute to the survival of both macrophages and microglia cells.

Conflicts of Interest

The authors declare no conflicts of interest.

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