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The Phytocannabinoid Cannabidiol Inhibits Migration and Reactive Oxygen Species Generation in Human Polymorphonuclear Leukocytes

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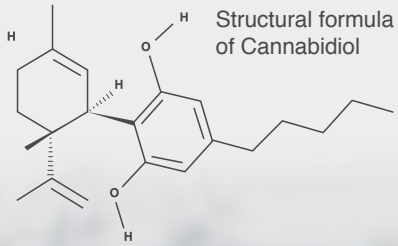
Background

Polymorphonuclear leukocytes (PMN) are the first line of the host defence, being involved in antigen recognition, inhibition of pathogen spreading throughout the body and clearing the invading cells. They carry out these functions through migration into the sites of infection, phagocytosis of the opsonized pathogens, release of neutrophil extracellular traps (NETs) and production of pro-inflammatory mediators including cytokines and reactive oxygen species (ROS). However, an inappropriate or exacerbate neutrophil response to invaders may contribute to tissue inflammation in a plethora of pathological conditions [1]. Cannabidiol (CBD) is a C21 terpenophenolic compound naturally occurring in significant amounts in the flowers of Cannabis sativa (English name: Cannabis). It exerts most of its pharmacological effects through interaction with the endocannabinoid system including the cannabinoid receptors type 1 (CB1) and type 2 (CB2), the endocannabinoids and their metabolic enzymes [2]. Circumstantial evidencecurrently suggests that PMN do express functional CB2 [3], however, the effects of CBD on PMN functions are not yet plainly elucidated.



Structural formula of Cannabidiol

Aim

To evaluate the effects of CBD on PMN functions including cell migration, ROS generation and cytokine production.

Materials and Methods

Isolation of human PMN
Human PMN were isolated from buffy coats of healthy donors by Dextran sedimentation followed by Ficoll-Paque Plus density-gradient centrifugation, as previously described [4].

Cell migration assay
PMN migration was investigated by the Boyden chamber assay according to our previous study [5]. In brief, after instrument assembly, CBD was added in the upper chamber to PMN alone and in the presence of IL-8 (10 ng/mL) in the lower chamber. Both chambers were separated by a 3 μm pore-sized filter. After a 90-min incubation at 37 °C, the filter was harvested, dehydrated, fixed, and finally stained with haematoxylin. PMN migration was then quantified by light microscopy measuring the distance (in μm) from the surface of the filter to the leading front of cells.

ROS production assay
Intracellular ROS production was assayed by use of the redox-sensitive dye C-DCFH-DA. In each experiment, CBD was added to PMN for 1 hour. Fluorescence was then measured over 30 min by means of spectrofluorimeter in PMN in resting conditions and after stimulation with fMLP (0.1 μM). ROS levels were expressed in fluorescence intensity in arbitrary units (AU).

Cytokine mRNA expression assay
Freshly isolated PMN were resuspended in RPMI medium and cultured for 1 h alone or in the presence of CBD. Further, fMLP (0.1 μM) was added to PMN for 3 h. Following incubation, cells were harvested, and cytokine mRNA was extracted and quantified by real-time PCR using assay-on-demand kits. Gene expression level in a given sample was presented as $2^{-\Delta Ct}$ where ΔCt = [Ct (sample) - Ct (housekeeping gene)]. Relative expression was determined by normalization to 18S cDNA.

Results

IL-8 (10 ng/mL) increased PMN migration [(mean±SEM) 33.29±1.41 μm vs. 12.50±0.61 μm in resting cells, n = 7, P<0.001], and this effect was concentration-dependently reverted by coincubation with CBD **(Figure 1)**. In resting conditions, however, CBD did not affect PMN migration (data not shown).

ROS production was increased by fMLP (0.1 μM). Coincubation with CBD did not affect fMLP-induced ROS production (data not shown), however 1 h preincubation with CBD before fMLP stimulation reduced ROS production in a concentration-dependent manner **(Figure 2)**.

fMLP (0.1 μM) upregulated mRNA expression of IL-8, IL-6 and TNF-α. This effect remained unchanged during coincubation with CBD (data not shown), but it was concentration-dependently reversed when CBD was added 1 h before fMLP **(Figure 3)**.

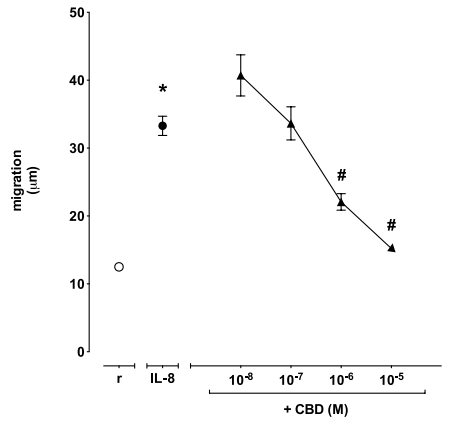


Figure 1. Effect of CBD on IL-8-induced PMN migration. Values are means±SEM (n = 7). * = P<0.001 vs resting and # = P<0.001 vs IL-8 alone by One-way ANOVA followed by Dunnett's Multiple Comparison Test.

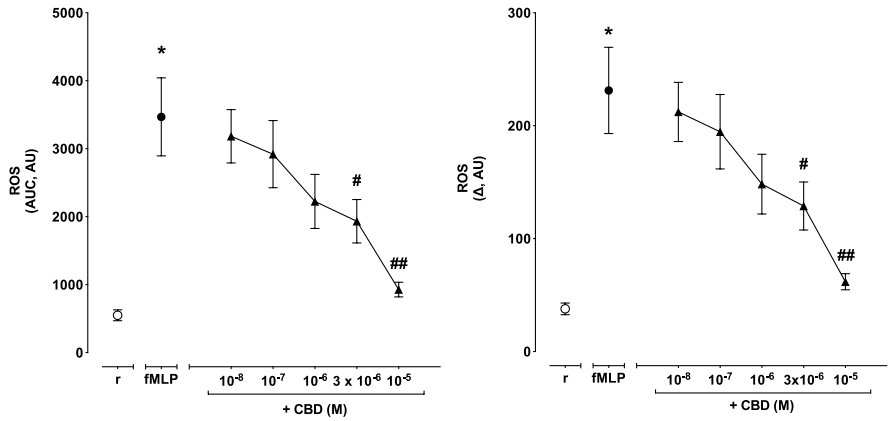


Figure 2. Effect of CBD on fMLP-induced ROS production in PMN. Values are means±SEM (n = 9). ROS changes were calculated over 30 min as the difference (Δ) between resting levels and peak levels induced by fMLP (left) and as the area under the ROS levels-versus-time curve (AUC) (right). * = P<0.001 vs resting, # = P<0.05 and ## = P<0001 vs fMLP alone by One-way ANOVA followed by Dunnett's Multiple Comparison Test.

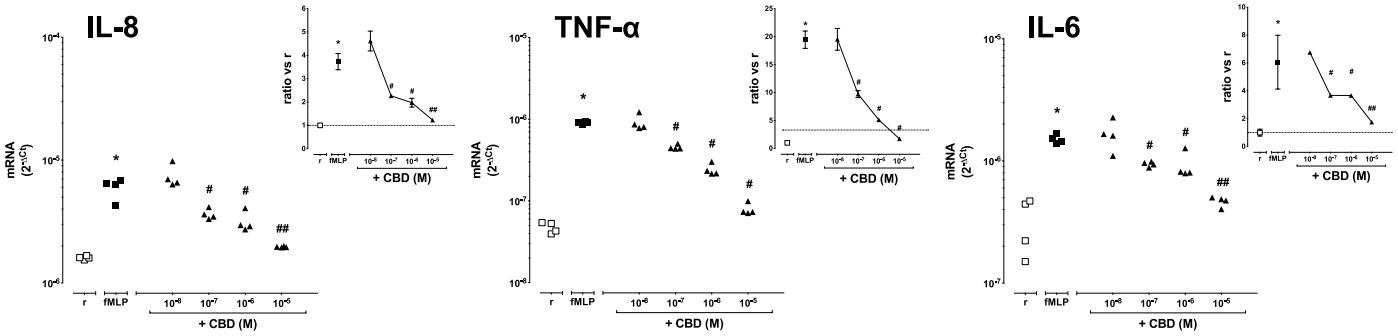


Figure 3. Effect of CBD on IL-8, TNF-α and IL-6 mRNA levels in PMN. Values are means±SEM (n = 4) and are shown as absolute values (main panels) and as ratio vs resting levels (insets). * = P<0.001 vs resting, # = P<0.01 and ## = P<0.001 vs fMLP alone by One-way ANOVA followed by Dunnett's Multiple Comparison Test.

Conclusions

CBD inhibits PMN functions including migration, ROS generation and proinflammatory cytokines production. Taken together, these findings suggest potential applications of CBD in the treatment or prevention of inflammatory disorders involving PMN. However, further research is required to unravel the receptor and signal-transduction mechanism underpinning the effects of CBD on PMN.

Reference

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