

Cannabidiol modulates migration and oxidative metabolism in human polymorphonuclear leukocytes

Alex MabouTagne¹, Massimiliano Legnaro¹, Gioela Ferrario¹,
Franca Marino¹, Barbara Pacchetti² and Marco Cosentino¹

¹ Center for Research in Medical Pharmacology, University of Insubria, Varese, Italy

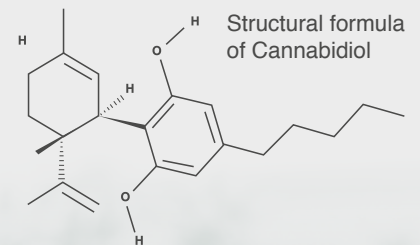
² Linnea SA, Riazino, TI (CH)

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 evidence currently suggests that PMN do express functional CB2 [3], however, the effects of CBD on PMN functions are not yet plainly elucidated.

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

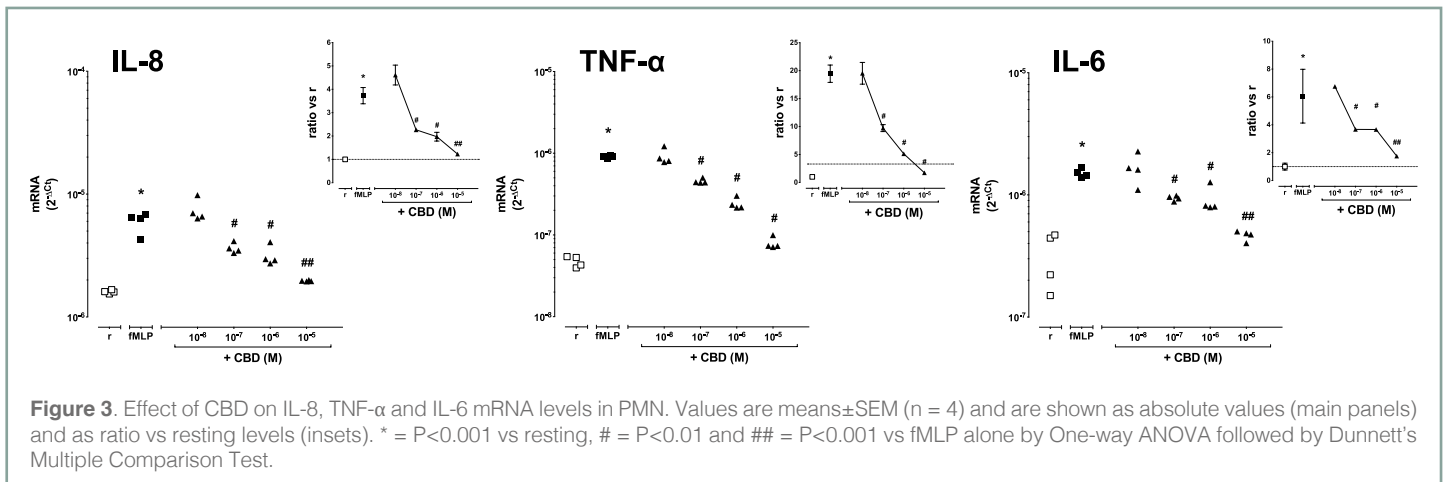
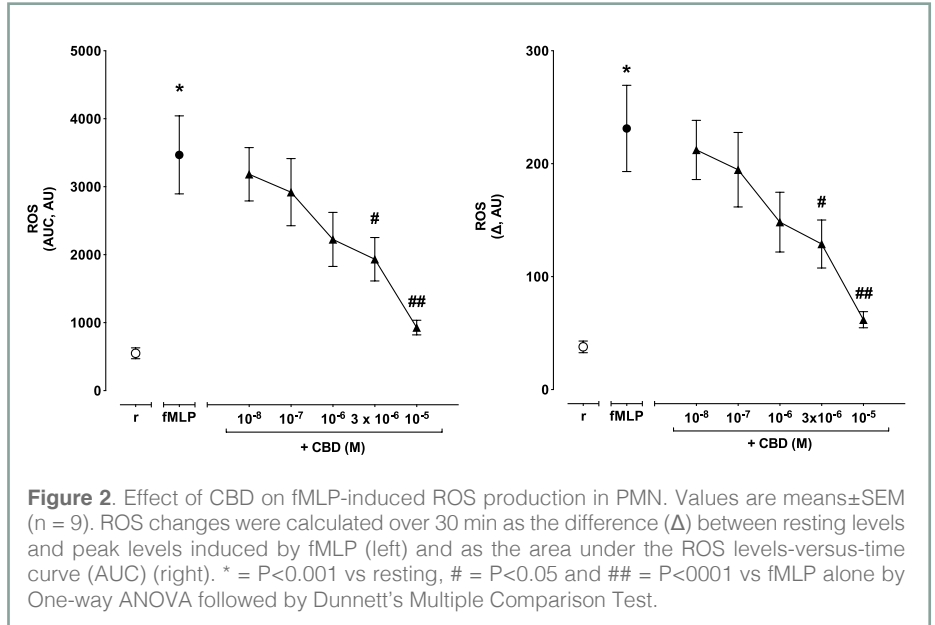
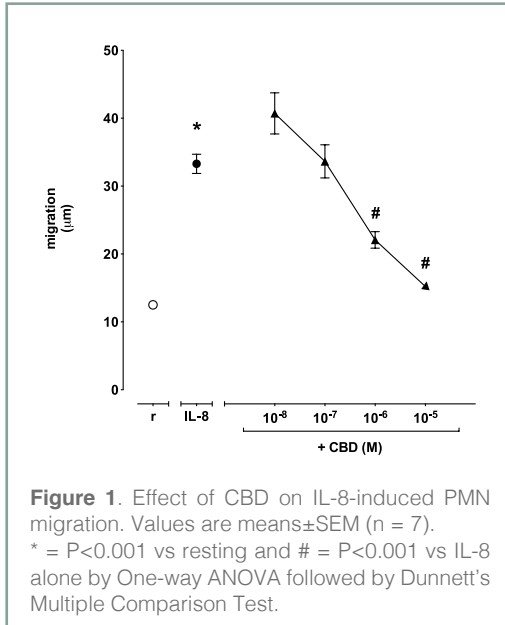
Freshly isolated PMN were resuspended in RPMI medium and cultured for 1 h alone or in the presence of CBD. Further, fMLP (0.1 µM) was added to PMN for 3 h. Following incubation, cells were harvested, and cytokine mRNA was extracted and quantified by real-time PCR using assay-on-demand kits. Gene expression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

Results

IL-8 (10 ng/mL) increased PMN migration [(mean±SEM) 33.29±1.41 μ m vs. 12.50±0.61 μ m 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 μ M). 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 μ M) 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**).



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

- [1] Wright HL, Moots RJ, Bucknall RC, Edwards SW. *Rheumatology*. 2010;49(9):1618-1631.
- [2] Pisanti S, Malfitano AM, Ciaglia E, et al. *Pharmacol Ther*. 2017;175:133-150.
- [3] Kurihara R, Tohyama Y, Matsusaka S, et al. *J Biol Chem*. 2006;281(18):12908-12918.
- [4] Cosentino M, Marino F, Cattaneo S, et al. *J Leukoc Biol*. 2000;67(5):637-643.
- [5] Maio RC, Cosentino M, Rossetti C, Molteni M, Lecchini S, Marino F. *Int Immunopharmacol*. 2011;11(2):194-198.

Grant

The research was supported with contribution of Linnea SA, Switzerland

LINNEA SA

Via Cantonale, CH-6595 Riazzino (TI), Switzerland - Tel. +41 (0)91 850 5050 - Email:info@linnea.ch