Comparison of a *Cannabis sativa L.* extract and its Derivative Cannabidiol on Human Polymorphonuclear Leukocyte Function

Alex Mabou Tagne[1], Massimiliano Legnaro[1], Alessandra Luini[1], Franca Marino[1], Barbara Pacchetti[2] and Marco Cosentino[1]

(1) Center for Research in Medical Pharmacology, University of Insubria, Varese, Italy, and (2) Linnea SA, Riazzino, TI (CH)

Background

Cannabis (*Cannabis sativa L.*, fam. Cannabaceae) is widely used for medical purposes. Several reports support that cannabis and its derivative cannabidiol (CBD) offer significant therapeutic benefits for a wide scope of pathological conditions. Among them, the clinical issues rooted in inflammation do stand out, nonetheless, the underlying mechanisms are not yet fully understood. Circumstantial evidence suggests that polymorphonuclear neutrophil leukocytes (PMN) are involved in the anti-inflammatory effects of cannabis.

Aim

To assess the effects of a cannabis extract on human PMN functions, including cell migration, oxidative metabolism and production of proinflammatory cytokines. We then sought to investigate whether such effects could be ascribed to its CBD content.

Materials and Methods

Test substances - *Cannabis sativa L.* extract containing 5% CBD (CM5) and pure CBD were kindly provided by LINNEA SA. Stock solutions were prepared in dimethylsulfoxide (DMSO, Sigma) at concentrations of CM5 50 mg/mL and CBD 10^{-2} M, and further diluted in either Hanks' Balanced Salt Solution (HBSS) modified with 10 mM HEPES (HBSS/HEPES) or RPMI medium to obtain final concentrations (CM5 0.05-50 µg/mL and CBD 10^{-8}-10^{-5} M).

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 [1].

Cytotoxicity assays - Cytotoxicity of test substances was assessed on PMN by means of the MTT reduction method [2]. In short, PMN were resuspended at 1 × 10^6 cells/ml in RPMI medium supplemented with 10% FBS and 1% P/S. Cells were then seeded in duplicate in a 96-well round bottom plate (250 µl of suspension per well) and cultured for 24 h alone or in the presence of a test substance at 37 °C in 5% CO_2. The absorbance (optical density, OD, in arbitrary units) was measured using a microplate spectrophotometer.

Cell migration assay - PMN migration was investigated by the modified Boyden chamber assay according to our previous study [3]. Briefly, after instrument assembly, PMN at 1 × 10^6 cells/ml in RPMI medium were placed in the upper chamber alone or together with the test substance, while the lower chamber contained medium alone (spontaneous migration) or added with 10 ng/mL IL-8 or 0.1 µM fMLP (stimulated migration). 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.

ROS production assay - Intracellular ROS production was assayed by use of the redox-sensitive dye C-DCFH-DA as previously described [1]. Briefly, fluorescence was measured by means of a spectrofluorimeter. The effects of CM5 and CBD were tested on resting cells and on cells stimulated with 0.1 µM fMLP. In each experiment, C-DCFH-DA-stained PMN resuspended at 1 × 10^6 cells/ml in HBSS/HEPES were placed in the spectrofluorimeter and the test substances were added after a 60-s resting period.

IL-8, IL-6, TNF-α production assays - PMN were resuspended at 1 × 10^7 cells/ml in RPMI medium supplemented with 10% FBS and 1% P/S and placed in sterile 5-ml test tubes. Cells were then stimulated with LPS or 0.1 µM fMLP and cultured for 21 h alone or in the presence of a test substance at 37 °C in 5% CO_2. After incubation, cells were centrifuged and pellets and supernatants were harvested and stored at -80 °C. IL-8, IL-6, TNF-α gene expression was assessed from cell pellets by RT PCR as previously described [4]. IL-8, IL-6, TNF-α proteins were measured in culture supernatants using commercial ELISA kits according to the protocol supplied by the manufacturer.

Results

Cytotoxic effects of CM5 and CBD on resting PMN

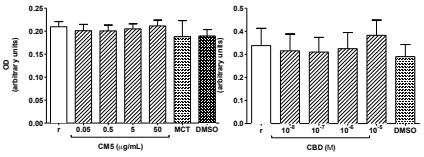

Figure 1. Results were expressed as mean OD value of duplicates. CM5 0.05-50 µg/mL (left panel) and CBD 10^{-8}-10^{-5} M (right panel) carried no meaningful effect on PMN viability in comparison to untreated cells.

Effects of CM5 and CBD on PMN migration

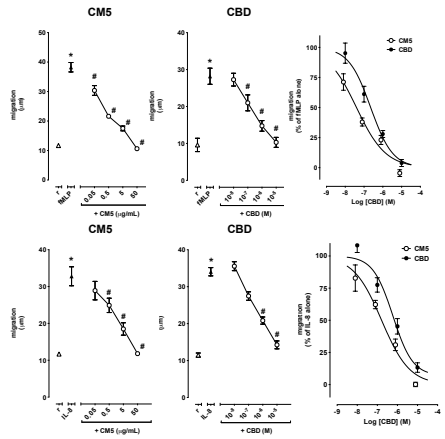

Figure 2. PMN migration was quantified by light microscopy measuring the distance (in µm) from the surface of the filter to the leading front of cells. PMN migration was increased by fMLP (upper panels) and IL-8 (lower panels). CM5 and CBD concentration-dependently reverted the effect of fMLP and IL-8 down to control values with CM5 50 µg/mL and CBD 10^{-5} M (left and center panels). Comparison of the concentration-response curves of CBD and CM5 (expressed as molar concentrations of CBD) showed however that CM5 was significantly more potent than CBD on both fMLP- and IL-8-induced migration of PMN, as indicated by lower IC_{50} and the absence of overlapping between respective 95% CI of IC_{50}s (right panel). In resting conditions neither CM5 nor CBD affected PMN migration at any of the concentrations tested (data not shown).

Effects of CM5 and CBD on ROS levels

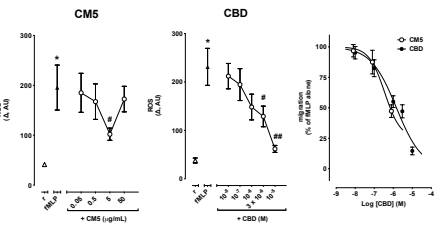

Figure 3. ROS changes, expressed as fluorescence intensity in arbitrary units (AU), were calculated as the difference (Δ) between resting levels and peak levels induced by the treatment. ROS production was increased by fMLP. Coincubation with CM5 up to 50 µg/mL or with CBD up to 10^{-5} M did not affect ROS production (data not shown). Preincubation for 1 h on the contrary resulted in a concentration-dependent attenuation of ROS production with both CM5 and CBD (left and center panels). CM5 however reached the maximum effect at 5 µg/mL, while CM5 50 µg/mL failed to modify the effect of fMLP (left panel). On the other side, CBD inhibited ROS production down to control levels with CBD 10^{-5} M (center panel). Comparison of the concentration-response curves of CBD and CM5 (expressed as molar concentrations of CBD) showed however that CM5 was slightly more potent than CBD, although the maximum inhibitory effect observed with CM5 5 µg/mL was still 25% of the effect of fMLP alone, while the maximum inhibitory effect of CBD 10^{-5} M completely suppressed the response to fMLP (right panel). In resting cells, CM5 did not affect ROS levels up to 5 µg/mL, however 50 µg/mL resulted in a huge increase of ROS production [from 59.9±4.0 AU in resting to 252.6±32.9 AU, n = 8, *P<0.001*]. On the other side, CBD did not significantly affect resting ROS levels, although CBD 10^{-5} M resulted in increased ROS production which however did not reach the statistical significance [71.4±10.4 AU in resting to 130.2±28.4 AU, n = 9, *P=0.1*].

IL-8, IL-6 and TNF-α production assays

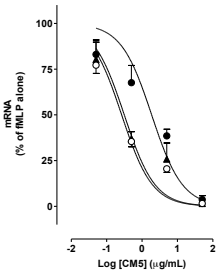

Figure 4. Effects of CM5 on IL-8, IL-6 and TNF-α mRNAs. mRNA was upregulated in fMLP-activated PMN and this was concentration-dependently restrained by CM5, reaching the maximum effect at 50 µg/mL. Noteworthily, CM5 was less active on IL-8 mRNA (IC_{50} = 1.9 µg/mL) than on IL-6 mRNA (IC_{50} = 0.3 µg/mL) and on TNF-α mRNA (IC_{50} = 0.3 µg/mL). In contrast, CM5 did not affect mRNA expression of all these genes in resting PMN (data not shown).

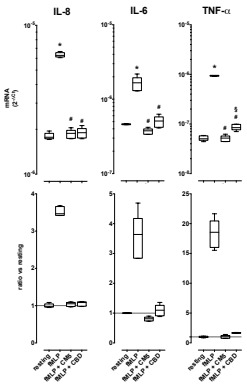

Figure 5. Effects of CM5 50 µg/mL on mRNA expression of IL-8, IL-6 and TNF-α, in comparison with CBD effects at the equimolar concentrations (10^{-5} M). As a result, both CM5 50 µg/mL and CBD 10^{-5} M reversed fMLP-induced mRNA expression of IL-8, IL-6 and TNF-α in a comparable extent. Interestingly, the CM5-induced inhibitory effect on TNF-α mRNA expression was more pronounced than that of CBD 10^{-5} M (*P*<0.05 vs CBD, n = 5).

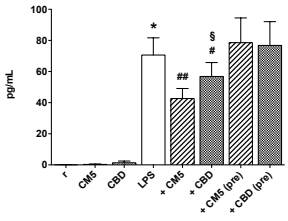

Figure 6. Effects of CM5 50 µg/mL and CBD 10^{-5} M on TNF-α production by LPS-activated PMN. TNF-α was increased by LPS 1 µg/ml (*P*<0.01 vs resting, n = 10). This effect was decreased by CM5 (*P*<0.01 vs LPS, n = 10) and CBD (*P*<0.05 vs LPS, n = 10). Interestingly, CM5 was more active than CBD (*P*<0.05, n = 10). Neither CM5 nor CBD were however active when added 1 h before LPS.

Conclusion

CM5 and CBD inhibit PMN functions, including migration, ROS generation and proinflammatory cytokines production. The effects of CBD and CM5 show however remarkable differences, suggesting that beyond CBD, other components of CM5 may contribute to its biological effects. In sum, such results support the use of cannabis and CBD as anti-inflammatory agents, however also warrant in-depth investigation of the underlying cellular and molecular mechanisms to better exploit their therapeutic potential.

References

1. *Cosentino M, Marino F, Cattaneo S, et al. J Leukoc Biol. 2000;67(5):637-643.*
2. *Andre CM, Hausman JF, Guerriero G. Front Plant Sci. 2016;7:19.*
3. *Maio RC, Cosentino M, Rossetti C, Molteni M, Lecchini S, Marino F. Int Immunopharmacol. 2011;11(2):194-198.*
4. *Kustrimovic, N., Comi, C., Magistrelli, et al. J. Neuroinflammation 2018; 15:205.*

Correspondence to:
Alex MABOU TAGNE, PharmD
PhD Fellow in Clinical and Experimental Medicine and Medical Humanities
Center of Research in Medical Pharmacology,
University of Insubria
Phone: +39 0332 217410, Fax: +39 0332 217409 - E-mail:
amaboutagne@uninsubria.it

Acknowledgements

AMT is supported by a PhD fellowship in Clinical and Experimental Medicine and Medical Humanities at the University of Insubria. The authors wish to express their gratefulness to LINNEA SA (https://www.linnea.ch/) for providing them with test substances.