Dimethyl and monomethyl fumarate, and cannabidiol effects in neurons and microglia

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Background and Objectives

Dimethyl fumarate (DMF), an approved oral drug for multiple sclerosis (MS), is metabolized into **monomethyl fumarate (MMF)** in the intestine. *In vitro* studies have focused on DMF-induced activation of the antioxidant pathway Nrf2, which could confer neuroprotection, and on its anti-inflammatory effects in microglia. However, less is known about MMF effects. **Cannabidiol (CBD)** is a phytocannabinoid that mitigates the mouse model of MS. Although the precise mechanisms are not fully understood, CBD also presents antioxidant and anti-inflammatory properties. Accordingly, CBD could be relevant as an adjuvant therapy for DMF in neuroprotection.

The aim of this study is to compare the effects of DMF, MMF and CBD on neuroprotective and anti-inflammatory parameters in neurons and microglia.

Results

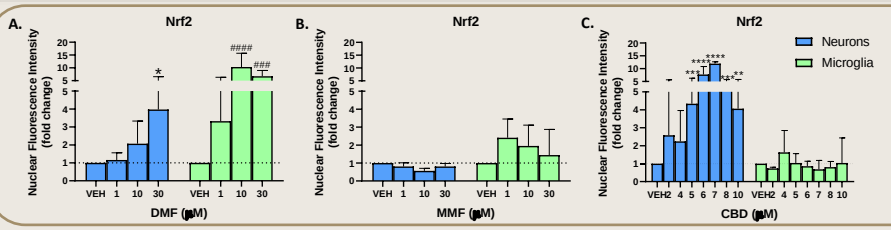

Figure 1. Nrf2 activation in neurons and microglia. (A) DMF activates Nrf2 both in neurons (10 μM) and in microglia (10 and 30 μM). **(B)** MMF does not activate Nrf2 neither in neurons nor in microglia at the same concentrations as DMF. **(C)** CBD (5-10 μM) activates Nrf2 in neurons, producing a higher induction than DMF, but does not activate Nrf2 in microglia. **(A-C)** Cells were treated for 4h with either vehicle (VEH) or drug. Data (mean ± SD) are representative of 3 different experiments. The Kruskal-Wallis test was used to determine statistical significance compared to vehicle control. *p<0,05 in neurons; #p<0,05 in microglia

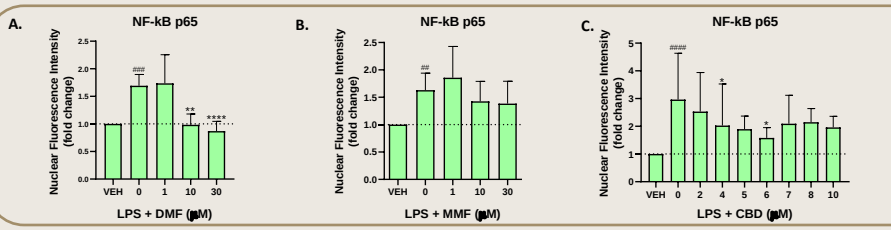

Figure 2. NF-kB p65 activation in microglia. (A) DMF (10 and 30 μM) inhibits LPS-activation of NF-kB. **(B)** MMF does not prevent NF-kB activation at the same doses as DMF. **(C)** CBD (4 and 6 μM) inhibits LPS-activation of NF-kB. **(A-C)** Microglia cells were activated for 0,5h and both treated with either vehicle (VEH) or drug. Data (mean ± SD) are representative of 3 different experiments. #p<0,05, using the Mann-Whitney to compare LPS with vehicle control. *p<0,05 using the Kruskal-Wallis test to determine statistical significance of the drugs compared to LPS.

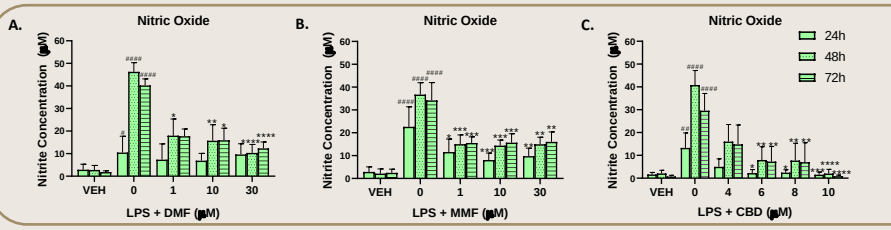

Figure 3. Nitric oxide (NO) production in microglia. (A) DMF (1-30 μM) reduces NO production at 48 and 72h. **(B)** MMF (1-30 μM) reduces NO production at 24, 48 and 72h. **(C)** CBD (6-10 μM) reduces NO production at 24, 48 and 72h. **(A-C)** Activated microglia cells were treated for 24, 48 or 72h with either vehicle (VEH) or drug. Data (mean ± SD) are representative of 3 different experiments. #p<0,05, using the Mann-Whitney to compare LPS with vehicle control. *p<0,05 using the Kruskal-Wallis test to compare the drugs with LPS alone.

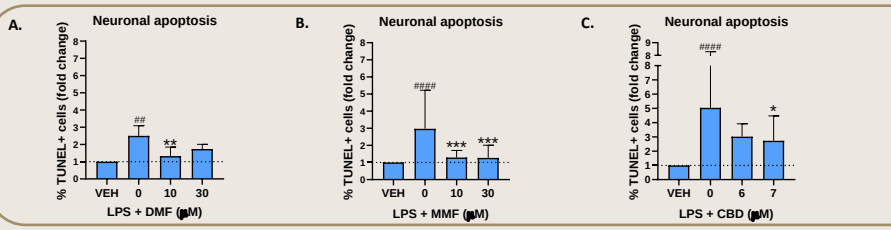

Figure 4. Apoptosis in neurons treated with microglia conditioned medium. (A) Neuronal apoptosis is reduced when activated microglia is treated with DMF 10 μM. **(B)** Neuronal apoptosis is also reduced when microglia is treated with MMF 10 and 30 μM. **(C)** Neuronal apoptosis is also reduced when microglia is treated with CBD 7 μM. **(A-C)** Neurons were cultured for 4h with microglia conditioned medium. Microglia had been previously treated with LPS and the drugs for 48h. #p<0,05, using the Mann-Whitney test to compare LPS with vehicle control. *p<0,05, using the Kruskal-Wallis test to compare the drugs with LPS alone.

Methods

- Primary neurons were obtained from the hippocampus of Wistar rat embryos on embryonic day 18
- Microglia cells were the BV2 murine cell line
- Cells were treated with either vehicle (VEH), DMF (1-30 μM), MMF (1-30 μM) or CBD (1-10 μM)
- For microglia activation, LPS 600 ng/ml was used in serum-deprived conditions
- Activation of Nrf2 and NF-kB p65 was considered as nuclear translocation, measured by confocal microscopy as the mean density of nuclear fluorescence. ToPro was used as a nuclear counterstain
- Nitric oxide (NO) production was measured using the Griess reagent system
- Apoptosis was detected by TUNEL assay using confocal microscopy

Conclusions

1. DMF and CBD share common mechanisms of action, with: (a) similar antioxidant properties in neurons (activation of Nrf2), (b) similar anti-inflammatory effects in microglia (inhibition of NF-kB p65 and nitric oxide production)
2. MMF, in contrast: (a) did not induce Nrf2 activation in neurons, (b) presented anti-inflammatory effects in microglia but through distinct pathways than DMF and CBD (independent of NF-kB p65), which should be further investigated

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