

ABSTRACT (300 words)

Neuroprotective and anti-inflammatory effects of dimethyl fumarate, monomethyl fumarate and cannabidiol in neurons and microglia

Short title (45 characters): DMF, MMF and CBD effects in neurons and microglia

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Background: Dimethyl fumarate (DMF), an approved 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.

Objectives: To compare the effects of DMF, MMF and CBD on neuroprotective and anti-inflammatory parameters in neurons and microglia.

Methods: Primary hippocampal neurons and the BV2 microglial cell line were treated with either vehicle, DMF (1-30 μ M), MMF (1-30 μ M) or CBD (1-10 μ M). BV2 were activated with 600 ng/ml LPS. Nuclear translocation of Nrf2 and NF-kBp65 was determined using confocal microscopy. Nitric oxide (NO) production was measured using the Griess reagent system. Apoptosis was detected using the TUNEL assay.

Results: DMF activated Nrf2 in both neurons and microglia, in contrast with MMF, which produced no activation. CBD produced a higher Nrf2 activation than DMF in neurons but no activation in microglia. DMF and CBD were also able of inhibiting NF-kB translocation in activated

microglia, as opposed to MMF. The three drugs reduced NO production in activated microglia. Neuronal apoptosis was also reduced when cultured with conditioned media from activated microglia treated with DMF, MMF or CBD compared to media from activated microglia alone.

Conclusions: DMF and CBD share common mechanisms of action, with similar antioxidant properties in neurons and anti-inflammatory effects in microglia. In contrast, MMF did not induce Nrf2 activation in neurons, and presented anti-inflammatory effects in microglia but through NF- κ B-independent pathways, in contrast to DMF and CBD, which should be further investigated.