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Phytocannabinoids Modulate Mitochondrial Dynamics in Cell Lines; Stress Adaptation

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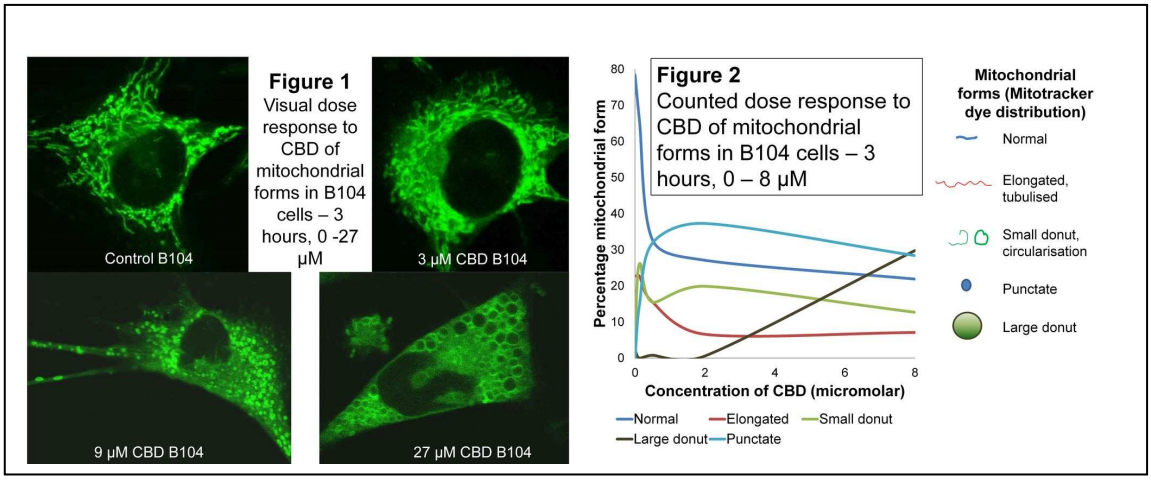
Phytocannabinoids are lipophilic phenolic compounds. They may locate to, and modulate, mitochondria [1,2,3,4].They can activate the intracellular stress response system [5], inhibit proliferation and induce autophagy and apoptosis [6,7]. Other polyphenols concentrate in mitochondria and induce mitochondrial biogenesis [8,9,10]. As the mitochondrial system is dynamic and responds to cell stress [11,12], studying its response to phytocannabinoids may be informative.

MB231, MCF10A, 3T3 and B104 cell lines were grown on 35 mm microscope dishes (Ibidi) until 30-60% confluence and pre-treated for 30 minutes with 50 nM Mitotracker dyes (Invitrogen), then exposed to vehicle or CBD, THC, THCV, CBDA and CBDV, for 0.5-48 hours. They were photographed using fluorescence confocal microscopy and 80-120 cells were assigned a mitochondrial phenotype if they had: > 10, normal; > 3, tubulised; > 10, punctate; > 3, small donut; > 0, large donut (vacuole). A T-test was used to compare groups for significance. FCCP and rotenone provided positive controls (0.1-10 µM).

From 0-27 µM, over 2-5 hours, THC, THCV, CBD & CBDV induced a dose-related loss of normal mitochondria, which became small donuts, then punctuate, then large donuts in mouse neuroblastoma B104s and 3T3 fibroblasts, and human breast cancer MB231 cells. At these doses CBDA did not induce large donuts, but was similar to FCCP and rotenone in inducing small donuts and punctuation in B104s. However, human normal breast epithelial MCF10a cells required 80 µM CBD to induce small donuts and punctuation. The figures display two dose response experiments. The data in figure 2 was analysed statistically, compared to control: loss of normal structure (2 & 8 µM, $p < 0.05$); small donuts (0.125-8 µM, $p < 0.01$); punctation (0.5-8 µM, $p < 0.01$); large donuts (8 µM, $p < 0.01$). After 48 hours, surviving cells exposed to phytocannabinoids still showed disturbed mitochondrial morphology.

As phytocannabinoids induce a biphasic response they may work via “hormesis”. Hormesis is a low dose stimulation, high dose inhibitory biphasic response to stress-inducing agents that involves mitochondria and improves cellular adaptability [13]. Thus, phytocannabinoid action could be explained by low dose receptor-based stimulation and high dose direct inhibition of both plasma and mitochondrial membrane function.



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