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An investigation into the effects of pharmacological modulation of omega-3 polyunsaturated fatty acids on endocannabinoid CB1 receptor signalling on regulating microglial cell fate

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Omega-3 polyunsaturated fatty acids (PUFAs) have an essential role in brain development and function, and there is increasing evidence that an elevated intake of the omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may confer benefits in a variety of neurological and neurodegenerative conditions. Furthermore, the omega-3 PUFAs have well characterised anti-inflammatory properties, such as decreasing the release of pro-inflammatory mediators from activated microglia. The endocannabinoid (eCB) system is a widely acknowledged key signalling pathway which down-regulates microglial activation. This pro-neurogenic activity is critical for the maintenance of brain homeostasis, as acute activation of local or systemic innate pro-inflammatory cascades has a profound negative effect on postnatal and adult neurogenesis. The aims of this study were therefore to investigate the effects of EPA or DHA on microglial cell fate, and the potential role of the eCB system, via CB1 cannabinoid receptor signalling pathways in these effects. The regulation of microglial cell proliferation by omega-3 PUFAs and the eCB system was investigated by BrdU ELISA analysis. Initially the effects of either EPA or DHA were assessed, and this was followed by an analysis of the potential interaction between these omega-3 PUFAs and the eCB system using AM251(1μM, t=24h), a potent CB1 antagonist. The addition of either EPA or DHA resulted in dose-dependent decreases in the number microglial cells (t=24h). EPA decreased the number of microglial cells by 10.9% at 0.5 μM, 21.3% at 5 μM and 37.2 % at 8 μM ($P< 0.01$ at 8 μM).Whereas, DHA decreased the number of microglial cells by 14.4% at 0.5 μM, 44.6% at 5 μM and 57.4% at 8 μM ($P< 0.001$ at 5 μM and 8 μM). These dose-dependent toxic effects were synergistically enhanced following pharmacological inhibition of the CB1 receptor. In the presence of AM251, EPA significantly decreased the number of microglial cells by 34.0% at 0.5 μM, 52.6% at 5 μM and 49.2% at 8 μM ($P=0.02$ at 0.5 μM and $P< 0.01$ at 5 μM and 8 μM). Whereas, DHA significantly decreased the number of microglial cells by 44.8% at 0.5 μM, 55.6% at 5 μM and 73.2% at 8 μM (all at $P< 0.001$). Since these effects were still evident in the presence of the AM251 alone, these results indicate that the toxic effects observed with both EPA and DHA were induced via a non-CB1 signalling pathway. It has previously been shown that EPA and DHA exert neuroprotective effects by inhibiting the release of pro-inflammatory mediators from activated microglia. Our data suggest an alternative explanation for these effects, as the observed decrease in pro-inflammatory mediators may be a secondary effect induced by omega-3 PUFA-induced microglial cell death, and that these effects are independent of CB1 signalling. By further elucidation of these distinct signalling pathways it may be possible to increase our understanding of the therapeutic potential of omega-3 PUFAs, and thereby improve treatment strategies in the emerging field of brain repair.

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