Eicosapentaenoic acid inhibits lipopolysaccharide (LPS)-induced nitrite production and cytokine release from J774 macrophages

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Background: Eicosapentaenoic acid (EPA), an omega-3 (ω-3) fatty acid, reduced cardiovascular (CV) events in high-risk patients (REDUCE-IT) but the mechanism is not fully understood. Activated macrophages, characterized by cytokine release and increased inducible nitric oxide synthase (iNOS) activity, contribute to atherosclerosis. As both a substrate for and potential inhibitor of cyclooxygenase (COX), EPA may reduce iNOS activity.
Purpose: The purpose of this study was to evaluate the dose-dependent effects of EPA on nitrite and cytokine release from lipopolysaccharide (LPS)-activated macrophages.
Methods: Murine J774 macrophages were pretreated with vehicle or EPA at 10, 20 and 40 μM for 2 h, then challenged with LPS at 1.0 μg/ml. After 24 hr, iNOS activity was measured by nitrite production using the Griess assay. EPA was compared to the COX inhibitor diclofenac at 1.0 μg/ml. Levels of interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) in cell supernatant were measured by immunochemistry using colchicine as a positive control.
Results: Activated macrophages caused a >4-fold increase in nitrite production ($p<0.001$) that was reduced by EPA in a dose-dependent manner. EPA decreased nitrite levels by 40, 62 and 77% at 10, 20 and 40 μM, respectively ($p<0.01$). Diclofenac separately reduced nitrite levels by 40% ($p<0.01$). EPA also reduced expression of IL-1β and TNF-α by 40% and 31%, respectively ($p<0.01$), in a manner similar to equimolar colchicine (10 μM). The reductions in IL-1β and TNF-α with EPA were dose-dependent.
Conclusions: EPA reduced macrophage activation as evidenced by decreased nitrite production and cytokine release similar to other anti-inflammatory agents. These findings indicate a novel effect of EPA on mechanisms of inflammation associated with vascular disease.