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Eicosapentaenoic acid inhibits oxidation of high density lipoprotein particles in a manner distinct from docosahexaenoic acid

Samuel C.R. Sherratt [a], R. Preston Mason [a, b, *]

[a] Elucida Research LLC, Beverly, MA 01915-0091, USA
[b] Department of Medicine, Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115-6110, USA

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ABSTRACT

The omega-3 fatty acid eicosapentaenoic acid (EPA) reduces oxidation of ApoB-containing particles *in vitro* and in patients with hypertriglyceridemia. EPA may produce these effects through a potent antioxidant mechanism, which may facilitate LDL clearance and slow plaque progression. We hypothesize that EPA antioxidant effects may extend to ApoA-containing particles like HDL, potentially preserving certain atheroprotective functions. HDL was isolated from human plasma and incubated at 37 °C in the absence (vehicle) or presence of EPA and/or DHA; 5.0 or 10.0 μM each. Samples were then subjected to copper-induced oxidation (10 μM). HDL oxidation was inhibited similarly by EPA and DHA up to 1 h. EPA (10 μM) maintained significant HDL oxidation inhibition of 89% (0.622 ± 0.066 μM MDA; $p < .001$) at 4 h, with continued inhibition of 64% at 14 h, vs. vehicle (5.65 ± 0.06 to 2.01 ± 0.10 μM MDA; $p < .001$). Conversely, DHA (10 μM) antioxidant benefit was lost by 4 h. At a lower concentration (5 μM), EPA antioxidant activity remained at 81% (5.53 ± 0.15 to 1.03 ± 0.10 μM MDA; $p < .001$) at 6 h, while DHA lost all antioxidant activity by 4 h. The antioxidant activity of EPA was preserved when combined with an equimolar concentration of DHA (5 μM each). EPA pretreatment prevented HDL oxidation in a dose-dependent manner that was preserved over time. These results suggest unique lipophilic and electron stabilization properties for EPA as compared to DHA with respect to inhibition of HDL oxidation. These antioxidant effects of EPA may enhance certain atheroprotective functions for HDL.

1. Introduction

Consumption of fish or fish oils rich in omega-3 fatty acids has been shown to be associated with reduced risk of cardiovascular disease [1,2]. Eicosapentaenoic acid (EPA – 20:5; n-3) is an omega-3 fatty acid shown to improve biomarkers of inflammation such as high-sensitivity C-reactive protein while reducing oxidized low-density lipoprotein (oxLDL) levels compared to placebo [3–7]. In the *Japan EPA Lipid Intervention Study* (JELIS), purified EPA (1.8 g/d) combined with statin treatment resulted in a significant reduction in major coronary events compared to statin treatment alone that was not predicted by reduction in triglycerides (TG) alone [8]. Indeed, in other studies adding other TG-lowering agents such as niacin and fibrates to statin therapy failed to reduce cardiovascular events or slow plaque progression compared to statin treatment alone despite large reductions in TG levels [9–12]. These differences between EPA and other TG-lowering agents may be due, in part, to the potent antioxidant effects of EPA, which may result from its distinct lipophilic and electron stabilization properties [13,14]. Docosahexaenoic acid (DHA – 22:6; n-3) is also a long-chain omega-3 fatty PUFA that has essential functions in neuronal and retinal cells by modulating membrane rafts, fluidity and signal transduction mechanisms [15,16]. These molecules associate with distinct regions or domains in biological membranes, which differentially modulate membrane structure-function relationships [17–19]. Due to its longer chain length and additional double bond, DHA also has different effects than EPA on membrane fluidity, cholesterol crystalline domains and rates of membrane oxidation [13,14,20].

In patients with coronary artery disease, treatment with EPA has been shown to improve HDL function. Specifically, HDL isolated from these patients showed enhanced cholesterol efflux and improved HDL activities, including antioxidant and anti-inflammatory effects [21]. In a recent study using isolated human

* Corresponding author. Elucida Research LLC, P.O. Box 7100, Beverly, MA 01915-0091, USA.
E-mail addresses: ssherratt@elucidaresearch.com (S.C.R. Sherratt), rpmason@elucidaresearch.com (R.P. Mason).

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endothelial cells, EPA-enriched HDL inhibited cytokine-stimulated VCAM-1 expression and increased resolvin E3 production [22]. The EPA-enriched HDL also enhanced cholesterol efflux [22]. Such HDL protection associated with EPA treatment may be due, in part, to inhibition of lipoprotein oxidation, which could result from its distinct lipophilic and electron stabilization properties. In particular, the lipophilic structure and molecular space dimensions of EPA may allow it to insert more efficiently into, and potentially function effectively within, lipoprotein particles and cell lipid membranes as compared to other TG-lowering agents and DHA. In this study, we tested the effects of EPA and DHA on rates of oxidation in isolated human HDL.

2. Materials and methods

2.1. Materials

EPA and DHA were purchased from Sigma-Aldrich (Saint Louis, MO). EPA and DHA were solubilized in ethanol to 1 mM under nitrogen atmosphere. The compounds were further diluted in ethanol or aqueous buffer as needed.

2.2. Isolation and oxidation of HDL

The HDL subfraction was isolated from the plasma of healthy volunteers by iodixanol density gradient centrifugation and adjusted to a final apolipoprotein A-100 concentration of 10 mg/mL [23]. Sample aliquots (200 μg apolipoprotein A) were incubated with EPA, DHA, or vehicle (ethanol) control (at concentrations indicated in figure legends) for 30 min at 37 °C in a shaking water bath. Lipid oxidation was initiated with 10 μM $CuSO_4$ and assayed at various time points for up to 14 h. Oxidation was monitored by spectrophotometric detection of thiobarbituric acid reactive substances (TBARS) as previously described [24].

2.3. Statistical analyses

Mean ± standard deviation was calculated for separate samples or experiments. Analysis of variance, followed by Student-Newman-Keuls multiple comparisons *post hoc* analysis, was used for comparisons between three or more groups. Differences were considered to be significant for probability values less than 0.05.

3. Results

3.1. Dose-dependent effects of EPA on human HDL oxidation

The results of our findings are summarized in Figs. 1–3. As shown in Fig. 1, EPA (10 μM) significantly inhibited HDL oxidation by 92% at 1.0 h (4.71 ± 0.148 to 0.384 ± 0.097 μM MDA; $p < .001$) and substantial inhibition continued through to the latest time point at 14 h (64% inhibition; 5.65 ± 0.06 to 2.01 ± 0.10 μM MDA; $p < .001$). By contrast, DHA lost all antioxidant activity by 4 h. At a lower concentration of EPA (5 μM), we observed 81% (5.53 ± 0.15 to 1.03 ± 0.10 μM MDA; $p < .001$) inhibition of HDL oxidation at 6 h, as shown in Fig. 2. As observed at the higher concentration, DHA was unable to sustain antioxidant activity with time, exhibiting a 50% reduction in MDA formation by approximately 2 h and losing all activity by 4 h (Figs. 2 and 3).

The activity of EPA was also observed at 5 μM when combined with an equimolar concentration of DHA (Figs. 2 and 3). The combination produced a reduction that was almost identical to EPA alone at 5 μM. We saw 81% (5.53 ± 0.15 to 1.03 ± 0.05 μM MDA; $p < .001$) inhibition of HDL oxidation at 6 h with the EPA/DHA combination. This demonstrates that the prolonged antioxidant activity of EPA does not appear to be diminished in the presence of DHA.

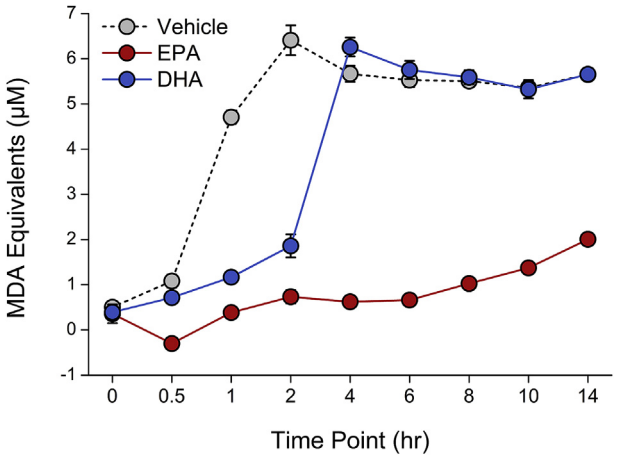

Fig. 1. EPA inhibited HDL oxidation in a prolonged manner as compared to DHA. Comparative effects of EPA and DHA, tested separately (each at 10 μM) on copper-induced oxidation of human HDL through 14 h. Values are mean ± SD (N = 3) and are expressed as molar equivalents of MDA.

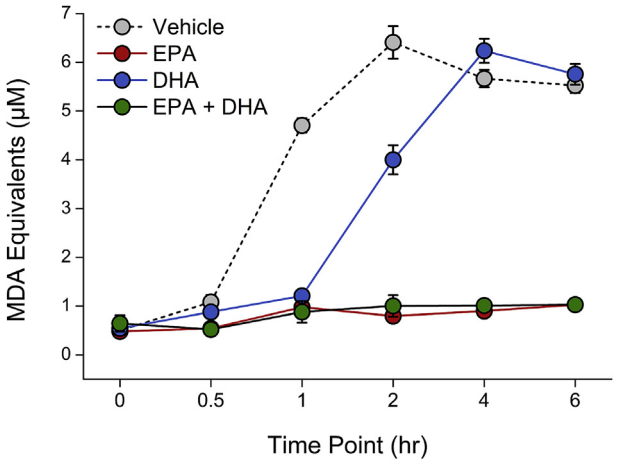

Fig. 2. EPA inhibited HDL oxidation in a prolonged manner as compared to DHA alone or in combination with EPA. Comparative effects of EPA and DHA, tested separately (each at 5 μM) and together (each at 5 μM), on copper-induced oxidation of human HDL through 6 h. Values are mean ± SD (N = 3) and are expressed as molar equivalents of MDA.

Finally, we observed that human HDL did not undergo auto-oxidation in the absence of copper when otherwise treated identically at 37 °C in a shaking water bath. Over 14 h, there was not a significant increase in HDL oxidation as compared to baseline (data not shown). This indicates that any increase in HDL oxidation observed in the above noted experiments is attributed solely to the addition of copper.

4. Discussion

In this study, EPA inhibited the oxidation of HDL at pharmacologically relevant treatment levels [25]. The sustained antioxidant effects of EPA on HDL oxidation were not reproduced by DHA under identical conditions. While DHA also interfered with lipoprotein oxidation at early timepoints, the effects were limited to a shorter time period as compared to EPA. We attribute such differences to the number of double bonds and carbon chain lengths, both of

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