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Reduced effect on apoptosis of 4-hydroxyhexenal and oxidized LDL enriched with n-3 fatty acids from postmenopausal women☆

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Abstract

Background: Oxidized low-density lipoprotein (oxLDL) promotes apoptosis in atherosclerotic plaques in the vascular wall, a process mediated through its oxidized lipids. 4-Hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE), derived from oxidation of n-6 and n-3 fatty acids, respectively, are among the major oxidized products in oxLDL.

Hypothesis: This study hypothesized that eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA)-rich versus linoleic acid-rich oxLDL obtained from postmenopausal women and HNE versus HHE differentially influence apoptosis in U937 cells.

Experimental Design: Thirty healthy postmenopausal women were supplemented with 14 g/day safflower oil (SO), 7 g/day of both fish oil and SO (low dose LFO) or 14 g/day fish oil (high dose HFO) for 5 weeks. Low-density lipoprotein, obtained after supplementation, was oxidized with 5 μM $CuSO_4$ at 37°C for 6 h. The concentration of cholesteryl ester hydroperoxides (CEOOH) and conjugated dienes was measured in the oxidized LDL (oxLDL). U937 cells were incubated with the oxLDL, 10 μM of HHE, 7 μM of HHE plus 3 μM of HNE, 5 μM of both HHE and HNE or 10 μM of HNE and the extent of apoptosis measured three ways.

Results: The concentration of CEOOH and conjugated dienes in oxLDL did not differ among the three treatment groups. The percent of apoptotic cells was approximately 40% lower when incubated with oxLDL obtained from the HFO-supplemented group than the SO-supplemented group measured by both the Annexin V and the DNA fragmentation assays ($P = .04$ and .004, respectively). Apoptosis of U937 cells was significantly lower in cells incubated with 10 μM of HHE, and mixtures of HHE and HNE than the 10 μM HNE when measured by the Annexin V, DNA fragmentation and 4,6-diamidino-2-phenylindole (DAPI) staining.

Conclusions: These data suggest that the cardioprotective properties of n-3 fatty acids may derive in part from their less reactive oxidized lipid metabolites.

Keywords: HNE; HHE; Postmenopausal women; oxLDL; Fish oil; Apoptosis

1. Introduction

Although cardiovascular diseases (CVD) are often thought of primarily as a problem for males, they also occur in women. In 2000, CVD claimed the lives of more women than men [1]. Data from observational studies suggested that hormone replacement therapy (HRT) in postmenopausal women reduced the risk of CVD by 35–50% [2–4]. Because of this, HRT was routinely prescribed to postmenopausal women. However, two placebo-controlled clinical trials published in the summer of 2002 changed the view of this widespread treatment. The Heart and Estrogen/Progestin Replacement Study II (HERS II) [5] found that HRT did not decrease the risk of CVD in women with previously diagnosed coronary artery disease (CAD). The Women's Health Initiative (WHI) trial [6], a primary prevention trial of HRT in postmenopausal women, also failed to demonstrate any benefit of HRT for the prevention of CAD. Indeed, the estrogen plus progestin arm of the study was terminated early because the women in this group demonstrated a statistically significant increase in breast cancer with the use of HRT. Despite the negative implications of the use of HRT, many women continue this therapy. Consequently, lifestyle modifications known to decrease CVD-related mortality are critically important to this group.

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Fish consumption, presumably through the highly unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is clearly cardioprotective [7–12]. The mechanism by which this occurs is not definitively resolved and is most likely multifactorial. A possible linkage may be through the effects of these fatty acids on apoptosis in the atherosclerotic plaque.

Atherosclerosis is the underlying condition involved in the pathogenesis of CVD. Apoptosis or programmed cell death is one of the processes that contribute to atherogenesis and plaque formation. Although various cell types undergoing apoptosis, that is, macrophages, smooth muscle cells and lymphocytes, have all been found in human atherosclerotic lesions [13–15], the dominant apoptotic cell population is macrophages. The impact of apoptosis of macrophages during the atherosclerotic process is ambiguous. Theoretically, it could lead to increased plaque stability due to decreased collagen breakdown [16]. However, a loss in the macrophage population could also diminish the scavenging efficiency of apoptotic bodies, resulting in increased plaque instability, rupture and thrombosis.

Oxidized low-density lipoprotein (oxLDL) is present in the atherosclerotic plaque [17] and is known to induce apoptosis in various cell types including smooth muscle cells, fibroblasts and monocytes/macrophages [18–21]. Numerous compounds found in oxLDL, including lipid hydroperoxides and several aldehydes, have been shown to cause apoptosis [22,23]. In a pilot study [24], we recently showed that oxLDL enriched in EPA and DHA induced less apoptosis in U937 cells, a human promonocytic cell line, than oxLDL enriched with linoleic acid. Further, our data suggested that this attenuated response was not associated with changes in the content of lipid hydroperoxides of the oxLDL.

The 4-hydroxyalkenals, 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE), are the most abundant aldehydes present in many tissues, body fluids and also in atherosclerotic lesions [25–28]. Hydroxynonenal and HHE are generated during the peroxidation of n-6 polyunsaturated fatty acid (PUFA) (i.e., linoleic acid and arachidonic acid) and n-3 PUFA (i.e., EPA and DHA), respectively. The differential effect of these aldehydes on induction of apoptosis has been poorly explored [29]. Consequently, the purpose of this study was twofold: first, to confirm in a clinical trial with postmenopausal women using HRT that EPA/DHA-enriched oxLDL decreased apoptosis of U937 cells compared to linoleic acid-enriched oxLDL; second, to determine whether HNE and HHE differentially influenced apoptosis in U937 cells.

2. Methods and materials

2.1. Subjects

Thirty postmenopausal women, between 51 and 71 years of age, were recruited from the Piedmont Triad in North Carolina. They were healthy, nonsmoking, normolipidemic postmenopausal females who were using HRT. The subjects were willing to maintain a stable weight, refrain from eating fish and take no nutritional supplements except calcium and vitamin D during the course of the study. The study protocol was reviewed and approved by the Institutional Review Board at The University of North Carolina at Greensboro, and written consent was obtained from each subject prior to beginning the study.

Subjects were asked to keep 3-day dietary records during the supplementation period. The nutritional content of the diets was analyzed using Food Processor Plus (version 7.1; ESHA, Salem, OR). Blood samples were collected before and after supplementation after an overnight fast of ≥ 10 h into tubes containing Na_2EDTA (1.5 mg/ml plasma). Plasma was separated by low-speed centrifugation at $1300 \times g$ for 15 min at 4°C. Samples were either analyzed immediately or appropriately aliquoted and stored at −80°C until processed.

2.2. Supplementation

Subjects were randomly assigned to one of the three supplementation groups. The safflower oil (SO) group was given 14 g/day of linoleic acid-rich SO, the low dose fish oil group (LFO) was given 7 g/day of both fish oil and SO, and the high dose of fish oil group (HFO) was given 14 g/day fish oil. Subjects took the oil supplements for 5 weeks. The SO was purchased in bulk from Arista Industries (Darien, CT) and the fish oil was kindly provided by Omega Protein (Houston, TX). Banner Pharmacaps (High Point, NC) encapsulated both oils.

The fatty acid profile of the oils was measured by gas chromatography using heptadecanoic acid (NuChek Prep, Elysian, MN) as an internal standard as described previously [30]. The intake of monounsaturated fatty acids (MUFA) provided by the supplements was similar in all three groups. The intake of saturated fatty acid (SFA), primarily palmitic acid, was 1.7 g in the SO group, 2.5 g in the LFO group and 3.4 g in the HFO group. There was no detectable amount of EPA and DHA provided by the SO supplement. The LFO supplement provided 0.59 g/day of EPA and 0.50 g/day of DHA while the HFO supplement provided twice these amounts (1.18 g/day of EPA and 1.00 g/day of DHA). The goal of the supplementation was to simulate intakes of EPA and DHA that could be achieved by the daily consumption of fatty fish. The LFO supplement provided amounts that could be obtained from approximately one serving of Chinook salmon and the HFO supplement provided the amount that could be obtained from two servings [31].

The antioxidant content of the three supplements was matched. To accomplish this, the vitamin E content of the oils was measured by normal phase high-pressure liquid chromatography (HPLC) [32]. Appropriate amounts of α-, δ- and γ-tocopherol (courtesy of ADM Nutraceuticals, Decatur, IL) were added to both oils. After these additions, the oils