Brain, Behavior, and Immunity 60 (2017) 136–141

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Oxidative stress predicts depressive symptom changes with omega-3 fatty acid treatment in coronary artery disease patients

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ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form 23 September 2016
Accepted 8 October 2016
Available online 11 October 2016

Keywords:
Lipid peroxidation
Oxidative stress
Hydroperoxide
Malondialdehyde
Hydroxynonenal
Isoprostane
Omega-3 fatty acid
Fish oil
EPA
DHA
Eicosapentaenoic acid
Docosahexaenoic acid
Coronary artery disease
Heart disease
Cardiovascular
Depression
Mood

ABSTRACT

Background: Antidepressant efficacy of omega-3 polyunsaturated fatty acid (n-3 PUFA) treatment in coronary artery disease (CAD) patients remains unpredictable. N-3 PUFA can mitigate oxidative stress, which is common in CAD and may contribute to depressive symptoms. This study investigated whether greater pre-treatment oxidative stress, measured by the ratios of late-stage lipid peroxidation markers (malondialdehyde [MDA], 4-hydroxy-2-nonenal [4-HNE], and 8-isoprostane [8-ISO]) to an early-stage marker (lipid hydroperoxides [LPH]), predicted n-3 PUFA antidepressant benefits in CAD.

Methods: This was a secondary analysis of CAROTID (CAD Randomized Omega-3 Trial in Depression, NCT00981383). Patient demographics and medical characteristics were collected. Depressive symptoms were measured using the 17-item Hamilton Depression Rating Scale (HAM-D). Patients were then randomized to receive either 1.9 g/day n-3 PUFA or placebo for 12 weeks, after which HAM-D scores were reassessed. Baseline LPH, 4-HNE, 8-ISO, MDA and n-3 PUFA concentrations were analysed from fasting blood.

Results: Seventy-nine patients (age = 61.1 ± 8.5, 76% male, HAM-D = 7.5 ± 6.1) were included (n = 45 placebo, n = 34 n-3 PUFA). In the n-3 PUFA group, higher baseline ratios of MDA/LPH (primary analysis: $F_{1,33} = 6.20$, beta = −0.35, p = 0.018), 4-HNE/LPH (exploratory analysis: $F_{1,33} = 5.35$, beta = −0.32, p = 0.027), and 8-ISO/LPH (exploratory analysis: $F_{1,33} = 6.10$, beta = −0.33, p = 0.019), indicating higher oxidative stress, were associated with greater depressive symptom improvement. In each model, higher baseline EPA + DHA concentrations independently predicted depressive symptom improvement with n-3 PUFA (MDA/LPH: $F_{1,33} = 11.05$, p = 0.002; 4-HNE/LPH: $F_{1,33} = 11.36$, p = 0.002; 8-ISO/LPH: $F_{1,33} = 13.15$, p = 0.001). No associations were observed in the placebo group.

Conclusions: n-3 PUFA may be more likely to improve depressive symptoms in CAD patients with pre-treatment evidence of oxidative stress.

1. Introduction

Increased lipid peroxidation has been associated with the presence and severity of depressive symptoms (Mazereeuw et al., 2015). This may be particularly relevant to depressive symptoms among patients with coronary artery disease (**CAD**) given the involvement of oxidative stress in that condition (Negi and Anand, 2010).

In the early stage of lipid peroxidation, reactive oxygen species damage unsaturated lipids, producing lipid hydroperoxides (**LPH**). LPH may be neutralized by antioxidant defenses, or they may progress to later stages of lipid peroxidation if antioxidant defenses are overwhelmed (Forman et al., 2014). Late-stage lipid peroxidation markers include malondialdehyde (**MDA**), 4-hydroxy-2-nonenal (**4-HNE**), and 8-isoprostane (**8-ISO**), each of which has been previously associated with the presence of depressive symptoms (Mazereeuw et al., 2015).

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http://dx.doi.org/10.1016/j.bbi.2016.10.005
0889-1591/

Increased oxidative stress appears to be more closely related to depressive symptoms in patients with deficits in omega-3 polyunsaturated fatty acids (**n-3 PUFA**) such as eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) (Bigornia et al., 2016). Accordingly, n-3 PUFA have shown antioxidant effects against reactive oxygen species in clinical samples (Azizi-Soleiman et al., 2013; Lee et al., 2013), and these effects have been associated with reduced depressive-symptom like behaviour in animals (de Mello et al., 2014). As such, n-3 PUFA treatment of depressive symptoms may be particularly beneficial in patients with greater baseline evidence of oxidative stress; however, this has yet to be studied.

This study investigated whether the baseline severity of oxidative stress, as measured by the ratios of late-stage lipid peroxidation markers (MDA, 4-HNE, and 8-ISO) to an early-stage marker (LPH), was associated with improvement in depressive symptoms among n-3 PUFA treated CAD patients.

2. Methods

This was a secondary analysis of data from the CAD Randomized Omega-3 Trial in Depression (**CAROTID**), which was a randomized, double-blind, placebo-controlled trial investigating the antidepressant efficacy of 1.9 g/day n-3 PUFA treatment compared to placebo using a 12-week parallel arm design (Mazereeuw et al., 2016). This study was approved by the Research Ethics Boards of Sunnybrook Health Sciences Centre, University Health Network, and Trillium Health Partners, and was conducted according to the principles expressed in the Declaration of Helsinki.

2.1. Patients

Trial inclusion and exclusion criteria are detailed elsewhere (Mazereeuw et al., 2016). Briefly, patients enrolled in CAROTID were those with evidence of stable CAD (history of myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, or at least a 50% stenosis in one or more major coronary artery), aged 45–80 years, male or female, and the ability to speak and understand English. Excluded patients were those with a significant acute medical illness, clinically significant cognitive impairment, a neurological condition, unstable angina, or a contraindication to n-3 PUFA supplements. Antidepressant use was permitted if used at a stable dose for at least 3 months prior to the trial.

2.2. Design

Eligible patients consenting to participate in CAROTID were invited to a baseline visit, prior to treatment arm randomization. At baseline, patient demographic, anthropomorphic, medical, and medication information was documented. Whether or not a patient had previously experienced a depressive episode was also recorded.

Depressive symptoms were assessed using the 17-item Hamilton Depression Rating Scale (HAM-D) (Williams, 1988). Fasting (12 h overnight) blood was drawn and processed for analysis of serum lipid peroxidation markers and plasma n-3 PUFA. Patients were then randomized (1:1) to receive either 1.9 g/d n-3 PUFA supplements (1.2 g/d EPA + 0.6 g/d DHA + 0.1 g/d other n-3 PUFA) or placebo for 12 weeks. Depressive symptom severity was reassessed using the HAM-D at week 12.

2.3. Analysis of lipid peroxidation markers and n-3 PUFA

Serum concentrations of LPH were measured based on absorbance relative to hydroperoxide at 500 nm in spectrophotometry (Cayman; Item No. 705003). Serum concentrations of MDA (Cayman; item No. 700870) were measured based on the absorbance of reaction products with thiobarbituric acid reactive substances at 530 nm in spectrophotometry. Serum concentrations of 4-HNE (Cell Biolabs, Inc.; STA-338) and 8-ISO (Cayman; item No. 516351) were quantified by standard sandwich ELISA according to manufacturer's instructions.

Plasma EPA and DHA concentrations were measured by gas chromatography as previously described (Merino et al., 2011). The sum of baseline EPA and DHA concentrations (EPA + DHA, as a measure of plasma "omega-3 index" (Harris and Von Schacky, 2004)) were included as a planned covariate. All analyses were performed blinded to treatment allocation and patient characteristics.

2.4. Statistical analyses

Late-stage/early-stage ratios were calculated by dividing the concentrations of each of MDA, 4-HNE, and 8-ISO by the concentration of LPH, yielding the MDA/LPH, 4-HNE/LPH, and 8-ISO/LPH ratios. The MDA/LPH ratio was investigated in the primary analysis as MDA has been the most consistently measured marker in previous depression studies (Mazereeuw et al., 2015). The HNE/LPH and 8-ISO/LPH ratios were investigated in the exploratory analysis. The ratios were log-transformed to ensure consistent normality between them, and the resulting transformed values were used for analyses. Ratios of oxidative stress markers, including those comparing late-stage to early-stage lipid peroxidation, have been previously used to indicate the severity of oxidative stress (Scola et al., 2016; Andreazza et al., 2007).

The baseline MDA/LPH, 4-HNE/LPH, and 8-ISO/LPH ratios were assessed as predictors of depressive symptom change in both the n-3 PUFA and placebo groups using a repeated measures general linear model with HAM-D total score as the dependent variable with 2 observations (baseline and week 12). Predictive associations between those ratios and changes in depressive symptom scores over 12 weeks were also investigated using linear regression to provide context for the direction and increment of the association. Missing data were imputed using multiple imputation (Rubin, 1987). Planned covariates were age as well as baseline plasma concentrations of EPA + DHA due to their previously identified relationships with depressive symptom changes in CAD patients treated with n-3 PUFA (Carney et al., 2016) (Mazereeuw et al., 2016). *Post-hoc* analyses included exploration of the associations between each baseline ratio and depressive symptom changes with n-3 PUFA in a subgroup that completed the study protocol and a subgroup not using antidepressant maintenance medication. The presence of statistical outliers was determined using the interquartile range method and through visual inspection. Finally, the baseline ratios were deconstructed, and associations between individual lipid peroxidation markers and depressive symptom changes with n-3 PUFA were explored.

Statistical models were computed using SPSS statistical software, version 13.0, Chicago, IL, USA and all analyses were two-tailed.

3. Results

Between August 2010 and February 2014, 645 patients were assessed for CAROTID trial eligibility and 92 patients were enrolled into the randomization phase. As reported previously, compliance with n-3 PUFA supplements was good; however, n-3 PUFA treatment did not improve depressive symptoms over 12 weeks compared to placebo (Mazereeuw et al., 2016). Of the 92 patients enrolled in CAROTID, 79 patients (34 receiving n-3 PUFA and 45