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Elevated ratio of arachidonic acid to long-chain omega-3 fatty acids predicts depression development following interferon-alpha treatment: Relationship with interleukin-6

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ABSTRACT

Cross-sectional studies have found that an elevated ratio of arachidonic acid to omega-3 fatty acid is associated with depression, and controlled intervention studies have found that decreasing this ratio through administration of omega-3 fatty acids can alleviate depressive symptoms. Additionally, arachidonic acid and omega-3 fatty acids have opposing effects on inflammatory signaling. Exogenous administration of the inflammatory cytokine interferon-alpha (IFN-α) can trigger a depressive episode in a subset of vulnerable people, though associated risk factors remain poorly understood. Using a within-subject prospective design of 138 subjects, we examined whether baseline long-chain omega-3 (docosahexaenoic acid – DHA; eicosapentaenoic acid – EPA) and omega-6 (arachidonic acid – AA; di-homo-gamma-linolenic acid – DGLA) fatty acid status was associated with depression vulnerability in hepatitis C patients treated with IFN-α. Based on the literature, we had specific *a priori* interest in the AA/EPA + DHA ratio. Lower baseline DHA predicted depression incidence ($p = 0.04$), as did elevated DGLA ($p = 0.02$) and an elevated AA/EPA + DHA ratio ($p = 0.007$). The AA/EPA + DHA ratio predicted depression even when controlling for other critical variables such as sleep quality and race. A higher AA/EPA + DHA ratio was positively associated with both increasing Montgomery-Asperg Depression Rating Scores over time ($F = 4.0$; $p < 0.05$) as well as interleukin-6 levels ($F = 107.4$; $p < 0.05$) but not C-reactive protein. Importantly, omega-3 and omega-6 fatty acid status was not associated with sustained viral response to IFN-α treatment. These prospective data support the role of fatty acid status in depression vulnerability and indicate a potential role for omega-3 fatty acids in the prevention of inflammation-induced depression.

1. Introduction

Emerging evidence suggests that elevated systemic inflammation may contribute to the pathoetiology of major depressive disorder (MDD) (Dowlati et al., 2010; Howren et al., 2009; Raison et al., 2006; Zorrilla et al., 2001). Although there is accumulating evidence that a subset of MDD cases could be induced by inflammatory cytokines (Lotrich, 2012), most people are resilient to elevated inflammatory activity and do not develop MDD. For example, exogenous administration of inflammatory cytokines such as interferon-alpha (IFN-α) can trigger depression, but only in a subset (~30%) of patients (Capuron et al., 2002; Capuron and Miller, 2004; Lotrich et al., 2007; Musselman et al., 2001). While vulnerability factors for depression remain poorly understood, recent evidence has implicated interleukin-6 (Prather et al., 2009), poor sleep (Franzen et al., 2009), serotonin (Bull et al., 2008; Lotrich et al., 2009), and glucocorticoid resistance (Raison et al., 2010). Developing a better understanding of risk and resilience factors associated with inflammation-induced depression may provide novel targets for improving resilience.

Polyunsaturated fatty acids (PUFAs) play a critical influence in the regulation of inflammatory signaling and potentially vulnerability to MDD. The long-chain omega-6 fatty acid arachidonic acid (AA; 20:4*n*-6) is a substrate for the synthesis of prostacyclins, thromboxanes, and prostaglandins such as PGE_2. PGE_2 stimulates the synthesis of inflammatory cytokines (Portanova et al., 1998; Wang et al., 2010); and in turn prostaglandins may also be important in mediating the effect of peripheral inflammation on brain function. For example, inhibition of cyclooxygenase-2 (COX-2), the rate-limiting enzyme in the conversion of AA to PGE_2, can attenuate lipopolysaccharide (LPS)-induced increases in extra-cellular hippocampal serotonin (Linthorst et al., 1996). Moreover, adjunctive treatment with celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor,

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was found to augment the therapeutic efficacy of fluoxetine in MDD patients (Akhondzadeh et al., 2009). In contrast, dietary dihomo-γ-linolenic acid (DGLA, 20:3*n*-6), an *n*-6 fatty acid precursor of AA, can be converted via COX-2 to PGE_1 which has anti-inflammatory properties. Moreover, the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3), and their COX and LOX metabolites (E- and D-series resolvins) have anti-inflammatory and inflammation resolving properties (Calder, 2008; Hong et al., 2003). Therefore, the balance between these different omega-3 and omega-6 fatty acids play a critical role in regulating inflammatory homeostasis.

Prior cross-sectional studies have repeatedly observed deficits in long-chain omega-3 fatty acids (EPA and/or DHA) but not AA in patients with MDD, as summarized in a recent meta-analysis (Lin et al., 2010). Accordingly, the AA/EPA + DHA ratio is elevated in MDD patients and may be positively associated with depression symptom severity across a variety of studies (Adams et al., 1996; Conklin et al., 2007; Frasur-Smith et al., 2004; Maes et al., 1996; Tiemeier et al., 2003). Independent meta-analyses of controlled intervention trials have found that chronic dietary EPA + DHA supplementation, resulting in a reduction in the AA/EPA + DHA ratio, is associated with significant reductions in depression symptom severity in MDD patients (Freeman et al., 2006; Lin and Su, 2007). Prior prospective studies have found that low erythrocyte DHA levels are associated with depression during IFN-α treatment (Su et al., 2010) as well as future suicidal attempts in medication-free MDD patients (Sublette et al., 2006). Importantly, rodent studies have found that dietary-induced deficiencies in omega-3 fatty acids, and elevations in the AA/EPA + DHA ratio, result in increased inflammatory cytokine production (Kozak et al., 1997; Mingam et al., 2008; Song et al., 2003) and associated changes in central serotonin turnover (Kodas et al., 2004; McNamara et al., 2010a). Moreover, omega-3 fatty acid deficiency has been found to up-regulate omega-6 fatty acid biosynthesis (Hofacer et al., 2011; Igarashi et al., 2007) as well as the expression of COX-2 in rat brain (Rao et al., 2007); and supplementation with omega-3 fatty acids can reverse some of the inflammatory and behavioral effects of IL-1 in rodent models (Song et al., 2004).

In view of this evidence for a preliminary link between lower omega-3 fatty acid status and increased vulnerability to inflammation and depression, the present study prospectively investigated whether polyunsaturated fatty acid status was associated with an increased risk for developing depression in response to IFN-α treatment. We additionally examined whether the fatty acid profiles were associated with markers of inflammatory status, interleukin-6 (IL-6) and C-reactive protein (CRP), and whether fatty acid status influenced the ability of IFN-α to successfully resolve hepatitis C infection. Based on extant evidence reviewed above, our primary hypothesis was that low EPA and DHA levels at baseline, and specifically a higher AA/EPA + DHA ratio, would be associated with increased risk for developing depression during IFN-α treatment.

2. Methods

2.1. Participants and depression assessment

138 adult subjects (between ages 18 and 80) were examined for plasma fatty acids levels prior to IFN-α therapy. Subjects had to be recommended by a hepatologist for treatment of HCV with IFN-α. Exclusion criteria were active mood, anxiety, psychotic, or drug/alcohol use disorders within 6 months prior to starting IFN-α treatment – using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I); known neurological disease; or taking corticosteroids, antidepressants, anticonvulsants, and/or antipsychotics (although they could be taking as-needed sleeping medications). An overlapping subset of these subjects were previously examined regarding the relationship between IL-6 and depression (Prather et al., 2009). The study was approved by the University of Pittsburgh Institutional Review Board.

Of these 138 subjects, 99 eventually started weekly injections of pegylated (PEG) IFN-α2 (PEG-IFN-α2a: 135 μg/week or PEG-IFN-α2b: 120 or 150 μg/week) augmented with oral ribavirin. Prior to initiating IFN-α therapy, and monthly for four months after therapy was initiated, depression was assessed using both subjective and objective measures including the Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) and Beck Depression Inventory-II (BDI) as previously described (Franzen et al., 2009; Lotrich et al., 2009, 2007). Sleep quality was measured monthly using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). Criteria for Major Depression (MDD; via an abbreviated SCID-I) were assessed at baseline, if BDI > 15, within 48 h of any request by either the treating heptalogist or subject, or minimally every 2 months. Participants who developed MDD during the course of treatment – or where concerns about lethality arose – were typically started on an antidepressant, though some did discontinue IFN-α treatment. Within 16 weeks of IFN-α therapy, 41 subjects required some type of psychiatric intervention for severe mood problem (such as suicidal ideation and/or MDD).

2.2. Phospholipid fatty acid extraction and gas chromatography

Plasma from whole blood was obtained from all subjects between 10 AM and 4 PM prior to initiating treatment for hepatitis C (HCV), and stored at −80 °C until analysis. Folch reagent (2 mL Chloroform/Methanol 2:1) was added to 0.3 ml of plasma to extract the lipid layer, dried under nitrogen, and reconstituted with chloroform (100 μL). The lipid extract was then transferred to a reversed-phase packed SPE column (Alltech, Nicholasville, KY) and washed with chloroform (10 mL), to remove triglycerides, and then acetone (10 mL) to remove the cholesteryl esters. Phospholipids were then eluted with methanol (20 mL), and the combined methanol fractions evaporated. The sample was methylated using NaOH/MeOH (0.5 mL) and the derivatization was completed with BF_3/MeOH followed by heating for 15 min at 85 °C. To ensure total fatty acid methyl ester (FAME) extraction, NaCl (0.3 mL) was used before extraction with hexane. Sodium sulfate was added to the hexane layer to remove water, and the organic phase decanted and evaporated using nitrogen. Samples were then reconstituted with hexane (0.5 mL) and analyzed.

FAME's were analyzed using an HP 6890/5973 gas chromatograph/mass selective detector (Agilent Technologies, Santa Clara, CA). The column used to separate FAME's was an Agilent DB-FFAP 15 m × 0.1 mm with 0.1 μm of film thickness. Helium was used as carrier gas at a flow rate of 17.6 ml/min and a constant pressure of 53.8 psi. The initial temperature was set at 160 °C and increased after injection of 1 μl of sample to 240 °C at a rate of 15 °C per minute. Once the temperature of 240 °C was reached, it was maintained for 6 min for a total run time of 14.33 min. The transfer line was maintained at 280 °C and the filament at 70Ev for EI. The data were evaluated using a TIC for compound identification and SCAN mode to measure relative percent of each fatty acid. Fatty acid identification was based on retention times of authenticated fatty acid methyl ester standards (GLC 473B) and controls (GLC 462 and GLC 463) to ensure reproducibility (NuCheck Prep, Elysian, MN). Data are expressed as weight percent of total fatty acid pool (mg fatty acid/100 mg fatty acids). Our primary measures of interest were the two long-chain omega-3 fatty acids, DHA (22:6*n*-3) and EPA (20:5*n*-3), the two long-chain omega-6fatty acids AA (20:4*n*-6) and DGLA (20:3*n*-6), and the ratio of AA to DHA + EPA.