Omega-3 Fatty Acids in the Prevention of Interferon-Alpha-Induced Depression: Results from a Randomized, Controlled Trial

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Background: Interferon (IFN)-α therapy for chronic hepatitis C virus infection is frequently associated with depression. The routine prophylaxis with antidepressants might expose patients to adverse effects, hence, the need for alternative preventive interventions. Omega-3 polyunsaturated fatty acids are safe and effective essential nutritional compounds used for the treatment of depression, putatively through an anti-inflammatory action. In addition, lower erythrocyte levels of omega-3 polyunsaturated fatty acids have been associated with an increased risk of IFN-induced depression.

Methods: We conducted a 2-week, double-blind, placebo-controlled trial comparing eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and placebo for the prevention of IFN-α-induced depression. A total of 162 patients consented to participate and were randomized to the study. All of the patients completed the 2-week trial; 152 participants were followed throughout the 24 weeks of IFN-α treatment and were included in the analysis.

Results: Compared with placebo, the incident rates of IFN-α-induced depression were significantly lower in EPA-treated but not in DHA-treated patients (10% and 28%, respectively, versus 30% for placebo, $p = .037$). Both EPA and DHA significantly delayed the onset of IFN-induced depression (week of onset: 12.0 and 11.7, respectively, versus 5.3 for placebo, $p = .002$). EPA and DHA were both well tolerated in this population. EPA treatment increased both EPA and DHA erythrocyte levels, but DHA only increased DHA erythrocyte levels.

Conclusions: EPA is effective in the prevention of depression in hepatitis C virus patients received IFN-α therapy. Our study confirms the notion that anti-inflammatory strategies are effective antidepressants in the context of depression associated with inflammation.

Key Words: Chronic hepatitis C virus (HCV), clinical trial, omega-3 polyunsaturated fatty acids (n-3 PUFAs), inflammation, interferon-alpha (IFN-α), major depressive disorder (MDD)

Finding the best strategy to prevent neuropsychiatric adverse effects induced by interferon (IFN)-α will improve clinical outcome and shed light on the pathogenesis of inflammation-induced depression, but previous studies have had mixed results (see below), especially in patients who receive IFN-α for chronic hepatitis C virus (HCV) infection. Chronic HCV infection is a major public health issue and has a high rate of progression to liver cirrhosis and hepatocellular carcinoma (1,2). IFN-α is the standard therapy for HCV infection and will remain a cornerstone of therapy, even within the new combinations with ribavirin and protease inhibitors (3). However, the clinical impact of IFN-α is reduced by the common and severe neuropsychiatric adverse effects. For example, almost all the patients experience acute sickness behavior, including symptoms of fatigue, malaise, myalgia, arthralgia, anorexia, apathy, and cognitive impairment (4–7). In addition, up to 30% of patients develop IFN-α-induced depression (a major depressive episode according to DSM-IV diagnostic criteria) within the first 3 months (8–10). These neuropsychiatric side effects result in early discontinuation of IFN-α therapy and poor clinical outcome (11–13).

Because of the high rate of IFN-α-induced depression, there is an ongoing debate on the use of prophylactic antidepressant use (14). The use of antidepressants is supported by the frequently cited trial, in patients with malignant melanoma, demonstrating a significant preventive effect of paroxetine, a selective serotonin reuptake inhibitor (SSRI) (15). However, in patients with HCV infection, the prophylactic effects with SSRIs have been demonstrated by some (16–18), but not all (19–22), studies. Moreover, SSRI-induced gastrointestinal bleeding is a particular concern in patients with HCV infection (23), who may already have esophageal varices and low platelet count (24). In addition, the use of antidepressants in patients receiving IFN-α therapy has been associated with rare, but severe, adverse effects, such as retinal hemorrhaging and cotton-wool spots (15,25), bone marrow suppression, hepatotoxicity (21,26), and manic episodes (27). As most patients receiving IFN-α do not develop clinically significant depression, the routine pretreatment with antidepressant drugs might expose patients to unnecessary medications; it is thus important to find alternative strategies for the prevention of IFN-α-induced depression.

Omega-3 polyunsaturated fatty acids (ω-3 or n-3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential nutritional compounds with potential preventive and therapeutic effects against depression. Patients with major depressive disorder have lower levels of omega-3 PUFAs (28), and societies that consume a larger amount of omega-3 PUFAs have a lower prevalence of major depressive disorder (29,30).

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More importantly, meta-analyses and many clinical studies (31–33), if not all (34–36), have shown that omega-3 PUFAs have antidepressant effects. Taken together also with the evidence discussed below, these studies support the use of omega-3 PUFAs as an effective depression prophylactic strategy in at-risk groups, such as indeed patients taking IFN-α.

One of the hypothesized mechanisms underlying PUFAs' antidepressant effects is their neuroprotective and anti-inflammatory action (37,38). Indeed, EPA is important in regulating immune function by antagonizing membrane arachidonic acid (an n-6 PUFA), reducing prostaglandin E2 synthesis (39), and preventing the response to inflammatory stimuli (40–43). Moreover, omega-3 PUFAs have been found to have beneficial effects in animal models of cytokine-induced behavioral changes that resemble depressive behavior (44–46). Of particular relevance for the present study, we have recently demonstrated that lower DHA levels in the peripheral blood are associated with an increased risk of developing IFN-α-induced depression over the following weeks (9). We have hypothesized that this reflects less endogenous anti-inflammatory capability in those who later develop depression (9). Based on this and the other evidence discussed above, we have conducted this 2-week, double-blind, placebo-controlled trial to test the differential effects of the omega-3 PUFAs, EPA, and DHA against placebo in the prevention of IFN-α-induced depression.

We have specifically prescribed a short (2 weeks) intervention before IFN-α therapy, to potentially correct the lower omega-3 fatty acid levels that we had previously identified as a risk factor for the development of IFN-α -induced depression (9). Indeed, we also have measured the levels of PUFAs in the erythrocytes before and after the trial and correlated these with treatment response. In addition, we have chosen to test a prophylactic intervention that would be acceptable to most patients because of its brevity and because it would precede, and not overlap with, the IFN-α (and ribavirin) therapy. According to most studies, the active antidepressant component from omega-3 PUFAs is EPA (32,33), but we also wanted to test DHA because, as mentioned above, we have found that lower levels of this omega-3 PUFA predispose to IFN-induced depression (9).

Methods and Materials

Patient Selection

Since 2005, a psychiatric team has been working together with the hepatologists to provide an integrated care package for HCV patients referred for IFN-α therapy at the Liver Centre of China Medical University Hospital, Taichung, Taiwan, where the Institutional Review Board approved the study. In the period between July 2009 and June 2012, the hepatologists identified eligible HCV patients before they started the combination therapy with peginterferon α-2b (1.5 μg per kilogram of body weight once weekly) and ribavirin (1000–1200 mg daily). Patients were excluded from this study if they had a major depressive episode at the initial assessment; a lifetime history of psychotic disorders (e.g., schizophrenia or bipolar disorder); a history of alcohol or drug dependence within 1 year before entry into the study; and evidence of any unstable chronic medical conditions (e.g., cardiovascular, endocrine, hematological, renal, or neurological diseases). The diagnoses of psychiatric disorders were based on the structured Mini-International Neuropsychiatric Interview (47). All patients who agreed to participate in this study provided their signed written informed consent before enrollment.

Study Design and Recruitment

Two hundred seven patients with HCV were screened, 162 of them consented to participate and were randomized to the study, and all of them completed the 2-week trial; 152 participants were followed throughout the 24 weeks of IFN-α treatment and were included in the analysis. For allocation of the participants following simple double-blind randomization procedures, a computer-generated list of random numbers was used. The identical capsules were prepacked in bottles and consecutively numbered according to the randomization schedule by an independent nutritionist.

Figure S1 in Supplement 1 provides a flow chart summarizing study recruitment. Ten subjects discontinued the IFN-α treatment; they did not differ from the completers in any demographic features, including gender, age, married status, education years, and past history of depression. While the noncompleters did have significantly higher baseline scores than completers in depressive symptoms (Hamilton Rating Scale for Depression [HAMD]: 8.6 ± 3.65 versus 4.5 ± 4.49; $p = .018$) and neurovegetative symptoms (Neurotoxicity Rating Scale [NTRS]: 52.9 ± 41.74 versus 26.9 ± 29.58; $p = .010$), they were equally distributed among the three groups (EPA, $n = 4$; DHA, $n = 3$; placebo, $n = 3$).

The subjects were randomly assigned in double-blind fashion to EPA, DHA, or placebo, administered for 2 weeks before starting IFN-α therapy. Specifically, 2 weeks before the initiation of IFN-α therapy (week –2), patients started receiving a daily treatment of five identical capsules of EPA (3.5 g/day), DHA (1.75 g/day), or placebo (high oleic oil) in single or divided administration. The experimental capsules contained concentrated EPA (700 mg), DHA (350 mg), or high oleic oil (800 mg); they weighed 1000 mg, were deodorized with orange flavor, and were supplemented with tertiary-butyl hydroquinone (.2 mg/g) and tocopherols (2 mg/g) as antioxidants. The sources of EPA, DHA, and oleic acids were, respectively, anchovy fish body oil (purchased from AK BioTech, Ulsan, Korea), algal vegetable (purchased from DSM Nutritional Products, Basel, Switzerland), and safflower oil (purchased from Aarhus Karlshamn, Hull, England).

The recruited participants were evaluated at weeks –2 (when omega-3 fatty acid prophylactic intervention started) and 0 (when the prophylactic intervention stopped and IFN-α therapy started) and during weeks 2, 4, 6, 8, 12, 16, 20, and 24 of IFN-α therapy to assess the occurrence of major depressive episode with the structured Mini-International Neuropsychiatric Interview. Sociodemographic factors, including gender, age, education, and marital status, as well as the past psychiatric history, substance use history, and family psychiatric history, were recorded at the initial assessment. Severity of depressive symptoms and of neurovegetative symptoms were measured using, respectively, the 21-item HAMD (48), rated by trained psychiatrists, and the self-administered NTRS (49), both administered at weeks –2, 0, 2, 4, 6, 8, 12, 16, 20, and 24. The NTRS is a checklist questionnaire that has been frequently used for the evaluation of neuropsychiatric symptoms related to cytokine therapy; the items are categorized into general symptoms, nonpainful somatic symptoms, and painful somatic symptoms, with each item rated from 0 to 10 on a visual analog scale, and the final score ranging 0 to 390 (15,49–51). During IFN-α therapy, allowable concomitant medications included acetaminophen and other nonsteroidal anti-inflammatory agents for pain symptoms and fever; granisetron or ondansetron for nausea; lorazepam for severe anxiety; and zolpidem for insomnia. The results of routine biochemical laboratory examinations, the occurrence of adverse effects, and any reason for IFN-α discontinuation were recorded.

Laboratory Methods

Fatty acid composition of erythrocyte membranes was analyzed by thin-layer chromatography, and the level of individual fatty acid was measured with gas chromatography of methyl esters (Lipid Standards, FAMEs, Sigma Co., St. Louis, Missouri). Fatty acid profiles were identified by comparing the retention times with those of appropriate standard fatty acid methyl esters. The detailed step-by-step procedures have been published and described elsewhere (9,52,53). The levels of each fatty acid were expressed as a percentage of total fatty acids. Laboratory measures were conducted on coded samples by workers who were blind to subjects' information.

Data Analyses and Statistics

All participants who were recruited into the study (n = 162) completed the 2-week, double-blind, randomized-controlled trial; 152 participants were followed throughout the 24 weeks of IFN-α treatment and were included in the analysis. The primary outcome measurement of efficacy was the incidence of major depressive episode in the three groups at any time point during 24 weeks of IFN-α therapy, while the HAMD and NTRS were secondary outcome measurements. The power was calculated a priori: a sample size of 50 in each group would have had 80% of power (at $p < .05$) to detect a reduction of 10% in the risk of depression. The categorical data (occurrence of major depressive episode or not) were analyzed using χ^2 (chi-squared) test. Changes in HAMD and NTRS scores as a function of time were assessed using repeated measures analysis of variance using mixed linear modeling, and at each time point, the mean scores of HAMD and NTRS were compared among the three groups by analysis of variance and post hoc analyses when appropriate. Furthermore, Kaplan-Meier estimates, survival curves, and log-rank test were used to compare the cumulative time free from depression between groups. All probabilities were two-tailed, with p less than .05 considered statistically significant. We used the SPSS statistical software version 15 (SPSS Inc., Chicago, Illinois).

Results

Demographics

There were no statistical differences between the three groups (EPA, DHA, or placebo) in demographics (age, gender, education, and marriage status) (Table 1), psychiatric characteristics (past history of depression, baseline HAMD and NTRS scores) (Table 1), and HCV-relevant biological markers (alanine aminotransferase and HCV genotypes and HCV RNA titers) (Tables 1 and 2).

Efficacy Outcomes

As shown in Table 2, the incident rates of IFN-induced depression were significantly different among EPA, DHA, and placebo groups (10% vs. 28% vs. 30%, respectively; $\chi^2 = 6.62$, $p = .037$). The post hoc analyses showed that incident rate was significantly lower following EPA ($\chi^2 = 5.99$, $p = .014$) but not following DHA ($\chi^2 = .05$, $p = .8$), as compared with placebo. Both EPA and DHA significantly delayed the onset of IFN-induced depression as compared with placebo (mean 12.0 vs. 11.7 vs. 5.3 weeks, respectively; $F = 7.80$, $p = .002$).

Figure 1 shows the Kaplan-Meier survival curves of IFN-induced depression comparing EPA, DHA, and placebo groups. Log-rank tests showed a significant overall effect for treatment group ($\chi^2 = 6.74$, $df = 2$, $p = .034$). Specifically, subjects receiving EPA prophylactic treatment showed a lower risk of IFN-α-induced depression as compared with placebo treatment ($\chi^2 = 6.52$, $df = 1$, $p = .011$) or DHA treatment ($\chi^2 = 3.23$, $df = 1$, $p = .072$). However, there was no significant difference between DHA and placebo groups ($\chi^2 = .95$, $df = 1$, $p = .3$). The hazard ratios were 2.9 (confidence interval: 1.0–7.9, $p = .044$) for the comparison between EPA and DHA and 3.5 (confidence interval: 1.3–9.6, $p = .015$) for the comparison between EPA and placebo.

The HAMD and NTRS scores during the IFN-α therapy are shown in Figure 2A and 2B, respectively. The mixed model analyses showed significant effects on time of repeated measurements ($F = 63.8$, $df = 198$, $p < .001$ for HAMD and $F = 19.3$, $df = 184$, $p < .001$ for NTRS), confirming the IFN-α-induced changes over time, but there were no significant effects of group ($F = 1.4$, $df = 189$, $p = .2$ for HAMD and $F = .4$, $df = 153$, $p = .6$ for NTRS) and no group by time interaction ($F = 1.2$, $df = 198$, $p = .3$ for HAMD and $F = .7$, $df = 184$, $p = .8$ for NTRS).

As shown also in Table 2, participants in both omega-3 PUFA groups did not differ from those on placebo at weeks 0 and 24 during IFN-α therapy for either HAMD or NTRS. There were significantly lower HAMD scores in the EPA group only at week 4 (compared with placebo group; $p = .013$) and at week 8 (compared with both DHA and placebo; $p = .022$ and $p = .002$, respectively). However, there were no significant differences

Table 1. Demographics and Clinical Characteristics Among EPA, DHA, and Placebo Groups[a]

	EPA	DHA	Placebo	*p*
n	50	51	51	
Age (Years), Mean ± SD	53.1 ± 10.5	53.6 ± 9.4	52.3 ± 11.8	.81
Sex (Male), %	48%	49%	47%	.98[b]
Education (Years), Mean ± SD	10.3 ± 3.8	9.8 ± 4.3	10.5 ± 4.4	.63
Marriage (Married), %	96%	95%	89%	.95[b]
History of Major Depressive Disorder, %	4%	6%	8%	.72[b]
Psychological Assessments at Week −2				
Baseline HAMD, mean ± SD	4.4 ± 4.3	4.5 ± 5.0	4.6 ± 4.2	.98
Baseline NTRS, mean ± SD	27.6 ± 33.6	27.3 ± 29.7	25.8 ± 25.5	.95
Physiological Assessments at Week −2				
Alanine aminotransferase (U/L)	92.3 ± 59.6	106.9 ± 65.2	99.4 ± 61.6	.77
Type 1 HCV genotype (%)	72%	63%	60%	.45[b]

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HAMD, the 21-item Hamilton Depression Rating Scale; HCV, hepatitis C virus; NTRS, Neurotoxicity Rating Scale.

[a]The results are from analysis of variance unless otherwise specified.

[b]Indicates the results of χ^2 (chi-squared) tests.

Table 2. Primary and Secondary Outcome Measures Among EPA, DHA, and Placebo Groups[a]

	EPA	DHA	Placebo	*p*
IFN-Induced MDE, *n* (%)	5 (10%)	14 (28%)	15 (30%)	.037[b]
Time to MDE (Weeks), Mean ± SD	12.0 ± 5.8	11.7 ± 6.3	5.3 ± 3.2	.002
EPA Levels (%)				
Week −2	2.34 ± .68	2.43 ± 1.00	2.29 ± .92	.69
Week 0	3.08 ± 1.31	2.31 ± .73	2.47 ± 1.04	.017
DHA Levels (%)				
Week −2	4.37 ± 1.51	4.62 ± 1.08	4.34 ± 1.40	.81
Week 0	5.10 ± 1.33	5.85 ± 1.27	4.64 ± 1.39	.002
HAMD Score, Mean ± SD				
Week 0	4.5 ± 4.7	4.4 ± 4.8	4.4 ± 4.3	.93
Week 24	9.7 ± 6.2	12.1 ± 8.4	12.3 ± 7.3	.19
NTRS Score, Mean ± SD				
Week 0	30.2 ± 33.8	27.9 ± 25.7	28.9 ± 25.4	.92
Week 24	43.8 ± 35.8	49.4 ± 46.4	53.1 ± 45.8	.62
HCV RNA (≥200,000 IU/mL) (%)				
Week 0	78%	84%	78%	.32[b]
Week 24	4%	4%	6%	.87[b]

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HAMD, the 21-item Hamilton Depression Rating Scale; HCV, hepatitis C virus; IFN, interferon; MDE, major depressive episode; NTRS, Neurotoxicity Rating Scale.

[a]The results are from analysis of variance unless otherwise specified.

[b]Indicates the results of χ^2 (chi-squared) tests.

between the three groups in the HAMD at weeks 2, 12, 16, and 20 and in the NTRS at any week of assessment.

Fatty Acid Biomarkers

As shown in Table 2, there was no significant difference in DHA and EPA levels between the three groups before the trial intervention (at week −2). However, there were significant effects of EPA and DHA intervention on the PUFA levels at the end of the trial (week 0) (Table 2). Specifically, the EPA intervention significantly increased EPA (+32%, $p = .009$) and DHA levels (+17%, $p = .016$). The DHA intervention significantly increased DHA (+27%, $p = .002$) but not EPA levels (−5%, $p = .6$). When the three groups were compared at week 0, analyses of variance followed by post hoc tests found that the EPA intervention group had higher levels of EPA ($p = .002$ vs. DHA intervention and $p = .03$ vs. placebo groups), while the DHA intervention group had only higher levels of DHA ($p = .035$ vs. EPA intervention and $p = .001$ vs. placebo groups). The complete profile of erythrocyte composition in fatty acids is shown in Table S1 in Supplement 1.

In the overall sample, a total of 34 patients (22%) developed IFN-α-induced depression at some point during the 24-week therapy, while 118 (78%) patients did not develop IFN-α-induced depression. At week −2 (before the intervention), subjects who later developed IFN-α-induced depression had lower baseline EPA levels (2.00 ± .63) than those who did not (2.47 ± .92; $p = .031$) but not DHA levels (4.17 ± 1.02 for depressed vs. 4.53 ± 1.41 for nondepressed, $p = .28$). However, at week 0 (before IFN-α therapy, after the 2 weeks of omega-3 PUFA intervention), there were no significant differences in EPA or DHA levels between subjects who subsequently did or did not develop IFN-α-induced depression (EPA: 2.64 ± 1.16 for depressed vs. 2.50 ± .78 for nondepressed, $p = .6$; DHA: 5.22 ± 1.32 for depressed vs. 5.11 ± 1.64 for nondepressed, $p = .8$).

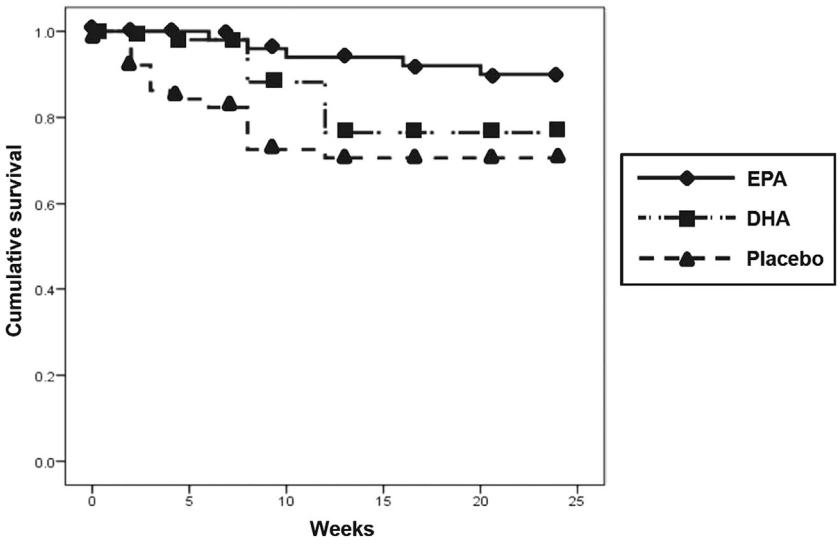

Figure 1. Survival curves (Kaplan-Meier estimates) of cumulative incidences of interferon (IFN)-induced depression of 152 patients with hepatitis C viral infection who were pretreated with eicosapentaenoic acid (EPA) ($n = 50$), docosahexaenoic acid (DHA) ($n = 51$), or placebo ($n = 51$) for 2 weeks before a 24-week IFN-α therapy. Using the log-rank test, we examined whether the curves displayed the risks of developing IFN-induced depression among EPA, DHA, and placebo groups were identical during a 24-week IFN-α therapy. Subjects that received EPA prophylactic treatment were associated with a significantly lower risk of IFN-induced depression as compared with placebo treatment ($\chi^2 = 6.52$, $p = .011$) and a trend of lower risk to DHA treatment ($\chi^2 = 3.23$, $p = .072$).

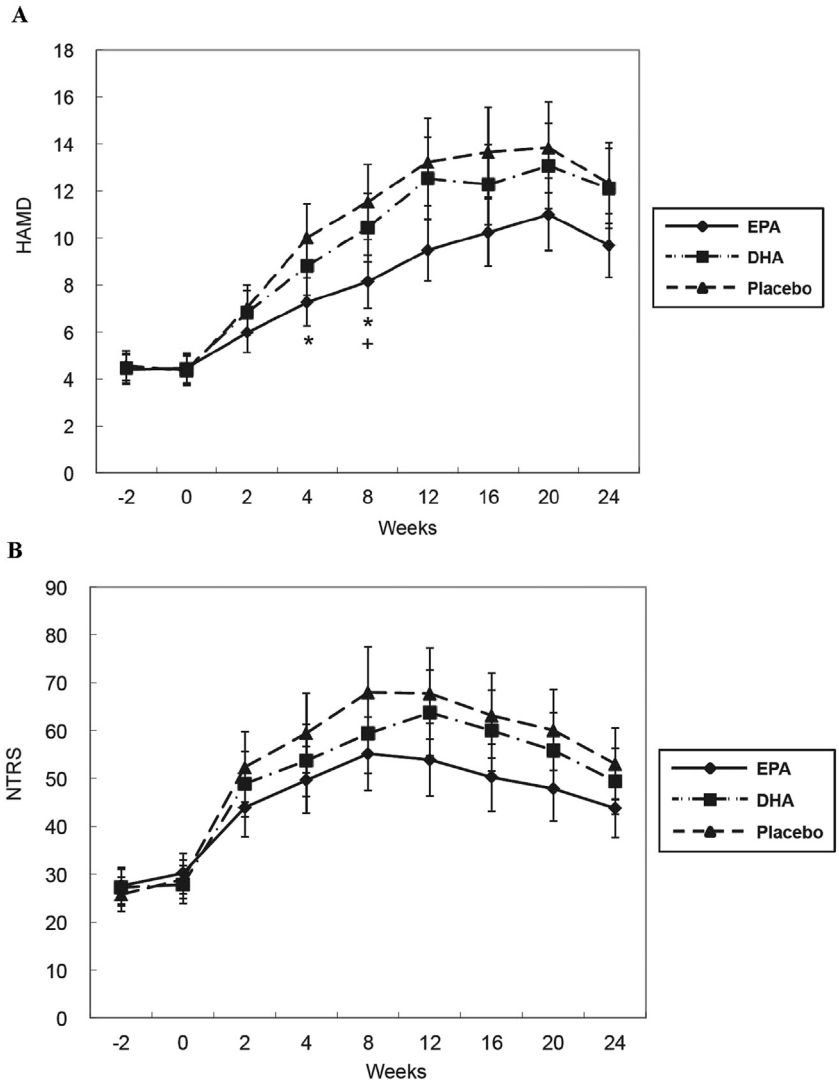

Figure 2. Changes in scores on the 21-item Hamilton Depression Rating Scale (HAMD) **(A)** and the Neurotoxicity Rating Scale (NTRS) **(B)** from baseline to week 24 among eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and placebo groups. Using a double-blind, placebo-controlled, randomized design, we examined the changes in HAMD **(A)** and NTRS **(B)** scores in a mixed-effects model for repeated measures analysis of 152 patients with hepatitis C viral infection who were pretreated with EPA ($n = 50$), DHA ($n = 51$), or placebo ($n = 51$) for 2 weeks before a 24-week interferon-α therapy. There was a significant effect of time with three groups exhibiting significant increases in HAMD **(A)** and NTRS **(B)** scores across interferon-α therapy. However, there was no main effect of treatment assignment and no treatment × time interaction. In HAMD **(A)**, subjects in the EPA group had lower HAMD scores at week 4 (than those of the placebo group*; $p = .013$) and at week 8 (than those of the placebo* and DHA groups+; $p = .022$ and $p = .002$, respectively). There was no significant difference in the NTRS **(B)** at weeks 2, 4, 8, 12, 16, and 20 among the three groups. The error bars indicate SEM.

Adverse Events

EPA and DHA were found well tolerated in this HCV population. No participant was withdrawn because of adverse events by investigators' decision, and reported events were all very mild and self-limited. There was no effect found in any blood laboratory parameter, such as abnormal bleeding time or liver function. In addition, there were no significant differences in the proportions of high HCV RNA levels (≥200,000 IU/mL) before and after IFN therapy among the three groups, showing no effects of the PUFA treatments on viral load.

Discussion

To our knowledge, this is the first study to demonstrate the beneficial effects of omega-3 fatty acids in the prevention of IFN-α-induced depression. The main finding is that EPA pretreatment significantly decreased the incidence of IFN-α-induced depression in HCV patients. In addition, both EPA and DHA pretreatment significantly delayed the onset of IFN-α-induced depression as compared with placebo pretreatment. Omega-3 fatty acids have been shown to have prophylactic effects in bipolar disorder (54,55), psychotic transition in ultra high-risk individuals (56), and the development of posttraumatic stress disorder following accidental injury (57). Therefore, our findings confirm and extend the notion that this nutritional intervention can have preventive effects in mental health and corroborate the evidence that anti-inflammatory strategies may have antidepressant effects, especially in the context of depression associated with inflammation.

The results of the current study support our previous findings showing that omega-3 PUFAs play a role in the risk of IFN-α-induced depression (9). In our previous study, we measured the erythrocyte levels of DHA and EPA and analyzed genetic variation in the phospholipase A2 (PLA2) and cyclooxygenase 2 (COX2) genes, the two key enzymes in the metabolism of omega-3 PUFAs. We found that participants with PLA2 BanI GG or COX2 rs4648308 AG genotypes have a higher risk of IFN-α-induced depression. In addition, the at-risk PLA2 polymorphism is associated with lower EPA levels and the at-risk COX2 polymorphism is associated with lower levels of both DHA and EPA during IFN-α therapy; furthermore, in the whole sample, having lower baseline DHA levels before IFN-α therapy is associated with a higher risk of developing depression during IFN-α therapy (9). This evidence is consistent with the present study, where we find that EPA pretreatment, increasing both EPA and DHA erythrocyte levels, is effective in preventing IFN-α-induced depression.

Previous clinical trials and meta-analyses have shown that the efficacy of omega-3 fatty acids might be dependent on the ratio of EPA and DHA (33) and have suggested that EPA, rather than DHA, might be the most active component of omega-3 PUFAs' antidepressant effects (31–33,58). Indeed, clinical trials using only DHA monotherapy as antidepressant strategy have shown conflicting findings: Marangell *et al*. (34) found no benefit over placebo for 2 g/day DHA, but Mischoulon *et al*. (59) found a dose-response effect supporting 1 g/day as superior to 2 g/day or 4 g/day, though the latter study was limited by the lack of a placebo arm. A recent meta-analysis has suggested that both EPA and DHA contribute to antidepressant effects but that the effects of EPA are stronger (60). Our current study, showing that EPA reduces the incidence of depression while DHA only delays the onset of depression, further supports this notion. Moreover, EPA can be metabolized into DHA, and EPA intervention can increase blood and brain levels of DHA (61), which is particularly relevant in this context, as we have identified lower endogenous DHA as a risk factor for IFN-α-induced depression (9). Indeed, it is interesting to highlight that the EPA intervention in our study increased both EPA and DHA levels. Incidentally, in our previous animal study, we demonstrated that a PUFA dietary intervention is able to increase PUFA levels in both erythrocytes and the brain, thus supporting the notion that PUFA changes measureable in the periphery reflect changes in the brain (62). These results therefore indicate possible synergetic effects of EPA and DHA on depressive symptomatology.

The anti-inflammatory action of EPA is likely to be particularly important in this context, where depression is triggered by an immune challenge. The prevailing model for IFN-α-induced depression points to a pivotal role of increased pro-inflammatory cytokines both in the periphery and in the brain (cerebrospinal fluid) of patients, with subsequent activation of the indoleamine 2,3-dioxygenase (IDO) pathway and the production of potentially depressogenic tryptophan metabolites, such as quinolinic acid (63). EPA has numerous anti-inflammatory properties by antagonizing membrane arachidonic acid formation, inhibiting COX2 enzyme activity, and reducing prostaglandin E2 synthesis (37). In turn, a reduction in COX2 activity not only has a general anti-inflammatory action but also can specifically down-regulate the IDO enzymes cascade and the production of its metabolites (64). Therefore, these findings confirm and extend recent studies showing that pro-inflammatory cytokines and the IDO cascade are involved in depressogenic mechanisms (65) and that high inflammation identifies depressed patients that are less likely to respond to standard antidepressants (66,67) and more likely to respond to anti-inflammatory drugs (68). In addition to this anti-inflammatory action, EPA and DHA may both exert their preventive effects also through neuroplasticity effects (69–71), which is a relevant molecular mechanism for antidepressant actions (72–74).

Our findings have direct clinical relevance for HCV patients receiving IFN-α. Although prophylactic effects of SSRI antidepressants have been studied in several randomized-controlled clinical studies (16–18,75), the use of such medications needs to be weighed against the risks of adverse effects and complications. Omega-3 fatty acid intervention, however, is a safe and well-accepted alternative antidepressant treatment (31,33,37). Omega-3 PUFAs have been shown to be well tolerated for patients with chronic medical illnesses and mental disorders (76), and adverse reactions are rare (77). It has been suggested that the potential antithrombotic effect of omega-3 PUFAs may theoretically increase the risk for bleeding: clinical trials have, however, shown that high-dose omega-3 PUFAs consumption is safe, even when concurrently administered with other agents that may increase bleeding, including aspirin and warfarin (78,79). Another potential safety concern is the susceptibility of omega-3 fatty acids to undergo oxidation, which may potentially contribute to toxicity; however, the conclusions are inconsistent (80), and adding antioxidant vitamin E to omega-3 PUFAs reduces oxidation.

In this study, we chose 3.5 grams of EPA and 1.7 grams of DHA per day, because our previous studies conducted in Taiwan using PUFAs as antidepressants showed the effective EPA dose to be between 2.2 g/day and 4.4 g/day and the effective DHA dose to be between 1.2 g/day and 2.2 g/day (53,81). These doses are relatively high, which is consistent with the fact that the baseline dietary content of fish is much higher in Taiwan than in many Western countries (30). We used an animal source for EPA and a vegetal source for DHA because of the availability; therefore, we cannot exclude that the reduced effectiveness of DHA might be linked to its vegetal source. The placebo contained 15% of linoleic acid, which may exert pro-inflammatory effects and theoretically worsen depression (76); however, the placebo also contained 75% of oleic acid, which could be converted to oleamide and potentially improve depression (82–84). Therefore, on balance, we believe that this was a truly inactive placebo.

The fact that EPA prevents the occurrence of depression without affecting the HAMD and NTRS scores of the whole group confirms the notion that EPA main effect is confined to subjects with clinically significant depression and does not extend to subjects with no or minimal depressive symptoms (33). Indeed, patients in this study developed clinically significant depression at any time points between week 2 and week 24 and the prophylactic effects are limited to around 20% of the sample and thus too diluted to statistically affect the scores of the whole group. It is worth highlighting, however, that Figure 2 clearly shows that the scores of both scales are numerically lower in the EPA groups than in the other two groups; moreover, HAMD scores are significantly lower in the EPA group compared with placebo at weeks 4 and 8 and compared with DHA at week 8. The effects of DHA are simply less strong than those of EPA, as shown by the fact that DHA delays the onset of depression: basically, it prevents depression up to week 10 (Figure 1), but the effects are not sustained. Obviously, it is possible that both EPA and DHA effects might have been more pronounced if the treatments had been continued beyond 2 weeks. Finally, this study is limited by its relatively small sample size, the lack of information on prescription of hypnotic agents, and the fact that noncompleters had higher depression scores at baseline, although they were equally distributed among the three groups.

In conclusion, EPA was beneficial for the prevention of IFN-α-induced depression, while DHA had only modest effects on delaying the onset. The data suggest that EPA or EPA/DHA combination is the best preventive strategy in this group of patients and potentially a suitable strategy for the wider pool of patients with depression associated with inflammation.

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Drs Su and Pariante created the concept. Drs Su, Lai, Yang designed the study, acquired the data, and take responsibility for the integrity and accuracy of the data. Drs Su and Pariante drafted and revised the manuscript. All the authors had full access to all the data in the study.

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ClinicalTrials.gov: N-3 Polyunsaturated Fatty Acids in the Prevention and Treatment for IFN-induced Depression; https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S0003K57&selectaction=View&uid=U0000KV0&ts=11&cx=-4rg5aa; NCT01620502.

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