Preliminary in vivo evidence of increased N-acetyl-aspartate following eicosapentanoic acid treatment in patients with bipolar disorder

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Abstract

Ethyl-eicosapentanoic acid (ethyl-EPA) may be beneficial in the treatment of bipolar disorder (BD) and may have a neurotrophic/neuroprotective role in patients with neuropsychiatric disorders. To investigate this we examined whether ethyl-EPA treatment of BD patients is associated with increased brain levels of N-acetyl-aspartate (NAA), a putative marker of neuronal integrity. Fourteen female BD outpatients with moderate depressive symptoms were administered 2 g of ethyl-EPA per day or placebo for 12 weeks in a randomized, double-blind fashion. Quantitative, proton magnetic resonance spectroscopy imaging data were obtained prior to randomization and after 12 weeks of treatment from a single 12 ml volume of interest centred above the body of the corpus callosum. A significant rise in NAA levels was observed in the ethyl-EPA treatment group compared with the placebo group (p=0.027). These results provide the first evidence for a probable neurotrophic role of ethyl-EPA treatment in BD underlining the need for more detailed investigation of its mechanism of action and therapeutic potential.

Keywords
bipolar disorder, omega-3 fatty acid, eicosapentanoic acid, magnetic resonance spectroscopy

Introduction

Bipolar disorder (BD) (American Psychiatry Association, 1994) often runs a chronic course associated with co-morbidity, suicide risk and psychosocial impairment (McQueen et al., 2001). Although a range of drugs, including antidepressants, antipsychotics, lithium and several anticonvulsants (valproic acid, lamotrigine), is available for the acute and/or prophylactic treatment of BD, some patients fail to respond or experience unacceptable side effects.

The need for new treatments has led several investigators to examine the potential role of omega-3 fatty acids, found in marine and plant sources, in the treatment of BD. This was based on the observation that omega-3 fatty acids, such as ethyl-eicosapentaenoic (ethyl-EPA), can affect the biophysical properties of phospholipid membranes (Armstrong et al., 2003), and interact with second messenger systems with similar effects to traditional mood stabilizers such as lithium (Stoll and Severus, 1996; Seung Kim et al., 2001). There have been four clinical trials looking at the effects of omega-3 fatty acid supplementation in unipolar depression and BD (Stoll et al., 1999; Nemets et al., 2002; Peet and Horrobin, 2002; Frangou et al., 2006), which have consistently shown significant symptomatic improvement following treatment with omega-3 fatty acids although methodology varied between trials.

Magnetic Resonance Spectroscopy (MRS) is based on the same principles as the more familiar Magnetic Resonance Imaging (MRI) and can be used to extract in vivo biochemical information from body tissue. Proton MRS (1H-MRS) spectra from the human brain can yield information about a number of biologically important molecules which include N-acetyl aspartate (NAA), a non-specific marker neuronal integrity/function; neural membrane constituents such as choline containing compounds (Cho); neuro-
transmitters and their metabolites such as glutamate and glutamine (Glx); cerebral sugars including myo-inositol (mI) and lactate (Lac) and creatine and phosphocreatinine (Cr, PCr).

'H-MRS has been used to study the effects of drugs on brain biochemistry in BD (Soares, 2003; Haldane and Frangou, 2004). Moore et al. (2000) found that lithium treatment resulted in increased NAA levels in various cortical regions in BD patients. They considered the observed increase in NAA levels supportive of a neurotrophic/neuroprotective role for lithium, first indicated by in vitro evidence of lithium-induced increase in the levels of the neuroprotective protein Bcl-2 (B-cell lymphoma/leukemia-2) (Manji et al., 1999). Evidence that ethyl-EPA can reduce or reverse brain atrophy has been reported in patients with Huntington’s Disease and unipolar depression (Puri et al., 2001; Puri et al., 2002). In the context of the above findings we conducted a preliminary proof of concept study to examine whether treatment with ethyl-EPA is also associated with increased cortical NAA levels.

Methods and materials

Subjects

Fourteen patients with BD type I disorder were randomized on a 1:1 basis according to a block balanced randomization code to receive either 2 g ethyl-EPA per day or placebo for a period of 12 weeks. The randomization codes were unblinded after the last patient had completed the last visit. Randomization was implemented by giving patients numbered containers containing soft gelatin capsules. All subjects were prescribed four identical-looking capsules daily, taken in two divided doses with food. Each capsule contained either 500 mg ethyl-EPA (purity >95%; supplied as LAX-101 by Llaxdale Ltd) or 500 mg liquid paraffin. Liquid paraffin is an inert compound commonly used as a lubricant laxative. Its usual laxative dose ranges between 15–30 g/day. Following randomization, patients were reviewed weekly and adherence to study medication was based on patient self-report and on pill counting. All patients remained on the same dose of lithium throughout the study and serum lithium levels were obtained at randomization and at study end point.

Inclusion criteria included: (a) female gender, (b) diagnosis of Bipolar Disorder I based on personal interview with the Structural Clinical Interview for DSM-IV Axis I Disorders (SCID; First et al., 2001), (c) a score of at least ten on the 17 items Hamilton Depression Rating Scale (HDRS; Hamilton 1960), (d) monotherapy with the same dose of lithium in the preceding 12 weeks.

Exclusion criteria included: (a) lifetime history of alcohol and substance abuse, (b) presence of suicidal ideation, (c) requiring imminent hospitalization, (d) history of poor treatment adherence, (e) any concurrent medical condition, (f) taking any prescribed drugs (including non-psychotropic medication) other than Lithium or contraceptives, (g) had taken fatty acids in the preceding 12 weeks, (h) history of head injury, (i) not on adequate contraception.

BD patients with moderate depression were selected for this study so they could be comparable in terms of phase of illness to BD patients included in the study of Moore et al. (2000) where increase in NAA was observed after 1 month of lithium treatment. As the potential of ethyl-EPA treatment to induce mania in BD patients has not been fully evaluated we considered it preferable to include patients who were on stable, chronic treatment with a ‘mood stabilizer’, of which the potential effect on brain NAA has only been studied for lithium. Finally, because of the preliminary nature of the study and the small sample size we choose to minimize potential gender-based variability by examining female patients only.

The study was approved by the Ethics Committee of the Institute of Psychiatry and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Participating patients were recruited by referrals from their treating physicians from secondary care. After complete description of the study written informed consent was obtained from all participants and signed agreement was obtained from their treating physicians.

Neuroimaging data acquisition and analysis

Subjects were scanned twice, using a 1.5 Tesla General Electric NV/i system, before being randomized, and then after 12 weeks of treatment with ethyl-EPA or placebo. Patients were clinically assessed weekly after enrolment and the HDRS scores used in subsequent analyses were obtained the day of each exam. The same acquisition protocol was used for both scans. Initially a coronal 3-D inversion recovery prepared fast GRASS T1-weighted dataset was acquired (TR=14 ms, TI=450 ms, TE=3 ms, flip angle=20 degrees, 1 data average, 256 × 256 × 128 matrix, 1.5 mm contiguous slices). Acquisition time was 6:27 minutes. A 12 ml voxel of interest (VOI) was placed in a standard location positioned immediately superior to the top of the centre of the corpus callosum (Fig. 1) and a PRESS spectrum (TE 35 ms, TR 3000 ms, 160 averages) obtained after CHESS water suppression. Special care was taken to place the VOI in identical locations for in the baseline (T1) and 12 week follow-up scan (T2) by.

Figure 1  Voxel placement of the volume of interest
references voxel position to readily identifiable anatomical gyral landmarks within the brain.

MRS data was analysed using LC-model software that uses a linear combination of model spectra to analyse the major resonances (Provencher, 1993). Concentrations of metabolites (Cho, Cr/PCr, NAA, mI and Glx) were determined using derived metabolite peak areas and documented relaxation characteristics of these neurochemicals at 1.5 Tesla. This protocol has been shown to be highly reproducible (Simmons et al., 1998).

**Statistical analysis**

Paired t-tests were used to compare the two treatment groups on clinical and demographic measures. For each metabolite, we conducted a repeated measures analysis. Pearson’s correlation coefficient was used to examine correlations between changes in MRS and clinical measures.

**Results**

Patients on placebo and ethyl-EPA group did not differ in mean age (41.5 ± 8.62 and 41.83 ± 4.66 years respectively, \( p = 0.93 \)), mean age of onset of BD (25.0 ± 3.67 and 27.43 ± 7.39 years respectively, \( p = 0.48 \)), mean lithium dose (667 ± 413.1 and 714 ± 254.5 mg respectively, \( p = 0.80 \)) or blood levels (0.85 ± 0.15 and 0.83 ± 0.19 mEq/L respectively, \( p = 0.91 \)) and remained virtually unchanged at study end point. At study entry the mean HDRS total scores for the placebo and ethyl-EPA groups were 11.83 ± 2.23 and 11.71 ± 3.35 respectively (\( p = 0.94 \)); at study end, the mean HDRS total scores for the placebo and ethyl-EPA groups were 12.00 ± 4.05 and 10.00 ± 4.28 respectively (\( p = 0.33 \)).

**Neuroimaging data**

Table 1 shows the composition of the volume of interest (VOI) for baseline (T1) and follow-up (T2) scans was examined in terms of mean grey and white matter and cerebrospinal fluid (CSF). No differences were found in the mean fractions of each tissue type between subjects at T1 and T2 and between groups at T1 and T2. Table 2 shows the mean levels of Cho, Cr/PCr, NAA, mI and Glx in the voxel of interest at baseline (T1) and follow-up (T2) scans. Comparison of the NAA levels between the two treatment groups using 2 \( \times \) 2 [time (T1, T2) \( \times \) treatment group] Repeated Measures Analysis of Variance showed a significant effect of time (\( F = 17.09, df = 1, 12, p = 0.001 \)) and a significant time by group interaction (\( F = 6.45, df = 1, 12, p = 0.27 \)), with the NAA being higher in the ethyl-EPA treated group at T2. No other significant differences were found. In order to detect differences between the two treatment groups at a level of significance \( p = 0.05 \) and 80% power we would have needed equal sample sizes for both groups of 92 for Cho, 11 for Cr/PCr, 36 for Glx and 1600 for mI. There were no significant correlation between lithium serum levels, HDRS scores and changes in any metabolite level in either group.

**Table 1** Mean (standard deviation) fraction of brain tissue in the volume of interest at baseline (T1) and follow-up (T2) scans

<table>
<thead>
<tr>
<th>Group</th>
<th>Grey Matter mean (sd)</th>
<th>White Matter mean (sd)</th>
<th>CSF mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.48 (0.07)</td>
<td>0.51 (0.54)</td>
<td>0.24 (0.10)</td>
</tr>
<tr>
<td>Ethyl-EPA</td>
<td>0.53 (0.72)</td>
<td>0.58 (0.26)</td>
<td>0.20 (0.11)</td>
</tr>
</tbody>
</table>

**Table 2** Mean (standard deviation) of metabolite levels in the voxel of interest at baseline (T1) and follow-up (T2) scans

<table>
<thead>
<tr>
<th>Group</th>
<th>Cho Mean (sd)</th>
<th>Cr/PCr Mean (sd)</th>
<th>NAA Mean (sd)</th>
<th>mI Mean (sd)</th>
<th>Glx Mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.68 (0.30)</td>
<td>1.80 (0.29)</td>
<td>7.63 (0.94)</td>
<td>8.19 (0.81)</td>
<td>10.92 (1.05)</td>
</tr>
<tr>
<td>Ethyl-EPA</td>
<td>1.79 (0.27)</td>
<td>1.85 (0.10)</td>
<td>7.91 (1.18)</td>
<td>8.07 (1.07)</td>
<td>10.79 (0.77)</td>
</tr>
</tbody>
</table>

NAA=N-acetyl-aspartate; Cho=choline containing compounds; Glx=glutamate and glutamine; mI=cerebral sugars including myo-inositol (mI) and lactate (Lac); Cr/PCr=creatine and phosphocreatinine

*significant difference, \( p = 0.027 \).
**Discussion**

In this study we found that in BD patients with moderate depression adjunctive treatment with ethyl-EPA over a 12 week period was associated with increased NAA levels compared to placebo. This increase was not linked to symptom improvement, as there was no correlation between depression and NAA levels.

Although in vivo neuroimaging techniques do not allow for the examination of events at cellular levels existing evidence suggests a number of potential pathways where EPA might act to increase NAA levels. NAA acts as an acetyl group donor for de novo neuronal membrane phospholipid synthesis (Tsai and Coyle 1995, Chakraborty et al., 2001). The observed effect of ethyl-EPA treatment could be linked to reduced neuronal membrane phospholipid turnover that has been associated with ethyl-EPA treatment in other studies (Puri et al., 2001; Puri et al., 2002). Another potential mechanism leading to NAA increase in the ethyl-EPA treated group is suggested by the action of ethyl-EPA on the transcription of the PLPL (proteolipid protein 1) gene, whose expression is found to be down regulated in BD (Tsai and Coyle 1995). The observed effect of ethyl-EPA on the transcription of the PLPL (proteolipid protein 1) gene, whose expression is found to be down regulated in BD patients (Tkachev et al., 2003). PLP makes up approximately 50% of CNS myelin and is thought to be essential for the compaction of the myelin sheath. Salvati et al. (2004) found that in rat glioma cells EPA up regulated PLP transcription via a cAMP-PKA cascade on the PLP gene promoter region. PLP transcription is also regulated through the peroxisomal proliferator activated receptor (PPAR) delta transcription factor (Bogazzi et al., 1994, Saluja et al., 2001). In vitro evidence suggests that EPA can bind directly to the PPAR delta ligand-binding domain and this may provide an alternative or additional mechanism for increased PLP gene transcription.

In this trial we saw no significant correlation between NAA rise and symptomatic change, which may be accounted for by the small sample size. It also suggests the possibility that treatment induced NAA changes are not associated with symptomatic change per se but may impact on other aspects of the pathophysiology of BD. Participating patients were on concomitant treatment with lithium that is also known to increase brain NAA levels (Moore et al., 2000). Since both the ethyl-EPA and placebo groups were similarly treated with respect to lithium dose and serum levels it is unlikely that the observed increase in NAA can be attributed to lithium treatment but the possibility of a synergistic action between lithium and ethyl-EPA cannot be excluded. The VOI approach used did not allow us to examine the regional distribution of NAA increase in the brain although on the basis of previous neuroimaging studies (Puri et al., 2000; Puri et al., 2002; Puri et al., 2004) regional specificity is not expected. The precise time course of ethyl-EPA associated NAA changes is not clear since we only collected data at two time points; our results however suggest that such changes are present at least 3 months after treatment initiation. It is also unclear whether more subtle changes are also present in other metabolites that could have been detected in a larger sample than that employed here. Based on our power calculations inclusion of a larger sample could have allowed us to detect a significant difference in Cr/PCr but not in the other metabolites.

This study provided preliminary evidence of ethyl-EPA induced NAA changes in the brain of patients with BD and is in need of replication. However, it suggests that further examination of the effects of ethyl-EPA on brain biochemistry and structure is worthwhile particularly given the popularity of such treatments with patient groups.

**Declaration of interest**

None

**References**


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