

Polyunsaturated fatty acid deficit in patients with bipolar mania

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Abstract

The aim of this study is to test the hypothesis that there is a depletion of polyunsaturated fatty acids of erythrocyte membranes in patients with bipolar disorder and to connect the previous therapeutic and psychoimmunological findings. Fatty acid compositions of erythrocyte membranes in 20 bipolar manic patients and 20 healthy controls were analyzed by thin-layer chromatography and gas chromatography. The major finding was significantly reduced arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) compositions in bipolar patients as compared to normal controls with *P* values of 0.000 and 0.002, respectively. There were no differences in total omega-3 and omega-6 polyunsaturated fatty acids. This abnormality may be related to the mechanisms of action of mood stabilizers and the previous findings on the abnormal psychoimmunology of patients with bipolar disorder. Larger sample sizes of medicated patients or drug-free manic, well-controlled designs on the diet and smoking, and fatty acid composition measurements during full remission after the index episode are warranted in future studies.

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1. Introduction

Several investigators (Stoll and Severus, 1996; Manji and Lenox, 1998) strongly suggested that the mechanism of action of mood stabilizers is involved in postsynaptic signal transduction processes. Two mood stabilizers (lithium and valproate) appear to treat the same symptoms of patients with bipolar disorder, through different effects on signal transduction in the brain. Recently, Chang et al. (2001) found that lithium and valproic acid have a common action in reducing turnover of arachidonic acid (AA), the major omega-6 polyunsaturated fatty acids

(PUFA) in rat brain. Furthermore, a 4-month double-blind, placebo-controlled study, comparing omega-3 PUFAs vs. placebo, in addition to usual treatment, suggested that omega-3 PUFAs may exhibit mood-stabilizing properties in bipolar disorder (Stoll et al., 1999). Thus, PUFAs, as well as lithium and antimanic anticonvulsants, seem to play an important role in the mechanism of mood stabilization by targeting parts of the "arachidonic acid cascade", which may be functionally hyperactive in mania (Rapoport and Bosetti, 2002).

It has been hypothesized that abnormalities in fatty acid composition may play a role in psychiatric disorders (Horrobin and Bennett, 1999). Maes et al. (1996, 1999) reported that patients with major depression had a significantly elevated ratio of eicosapentaenoic acid (EPA; 20:5n-3)/docosahexaenoic acid (DHA; 22:6n-3), lower level of EPA and total n-3 PUFAs, in both serum chole-

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teryl esters and phospholipids when compared to patients with minor depression and normal controls. Similar findings were revealed in terms of fatty acid compositions of the erythrocyte membrane (Adams et al., 1996; Edwards et al., 1998; Peet et al., 1998). Generally, PUFAs are classified mainly into omega-3 (or n-3) and omega-6 (or n-6) groups. Cerebral cell membranes are composed of certain PUFAs, which cannot be synthesized and must therefore be obtained from the diet. The abnormalities in PUFA composition in cell membranes can alter membrane microstructure, resulting in abnormal signal transduction and immunological regulation. In general, the omega-6 fatty acid, AA is proinflammatory and associated with up-regulation of release of various cytokines. In contrast, the omega-3 fatty acids, EPA and DHA, are anti-inflammatory (Horrobin and Bennett, 1999). Interestingly, patients with bipolar disorder have also been reported to have altered immune functions (Kronfol and House, 1988), including leukocytosis (Kronfol et al., 1988), higher prevalence of thyroid autoantibodies (Lazarus et al., 1986), high rate of immune-related diseases (Tsai et al., 1997), and abnormalities in cytokine levels (Maes et al., 1995; Su et al., 2002; Tsai et al., 1999, 2001). Thus, PUFAs also seem to play an important role in the abnormality of psychoimmunology of patients with bipolar disorder.

Despite this evidence from animal studies, psychoneuroimmunology studies, and clinical trials, well-controlled studies on PUFA levels in bipolar manic patients are still sparse. Mahadik et al. (1996) reported the only study that compared PUFA compositions of cultured skin fibroblasts of 12 schizophrenic patients to those of six bipolar patients and eight normal control subjects. They found that there was no difference between bipolar patients and normal subjects. However, their results are difficult to interpret due to various limitations. (1) The sample size is small ($n=6$) and the aim of their study is to examine the abnormalities of fatty acids in schizophrenic rather than bipolar patients. (2) The clinical status, such as durations of the illness, mood state (mania, depression or mixed), the severity of the symptoms, in these bipolar patients was not well-defined or controlled. (3) No information was given regarding to the use of the mood stabilizing drugs and the antipsychotic drugs, which were also found to influence the PUFA levels (Horrobin et al., 1997). (4) The PUFA level from fibroblasts (instead of erythrocyte) has not been confirmed to reflect that in the brain (Neuringer et al., 1984; Makrides et al., 1994).

To address these issues, we conducted a study on erythrocyte PUFAs in acute manic patients compared with matched normal controls.

2. Subjects and methods

Forty subjects (20 healthy volunteers and 20 patients with bipolar disorder) were recruited in this study. All of them were free of any medical illness, including immune

and endocrine disorders. Their ages were ranged between 18 and 65 years. The patients were admitted to the psychiatric ward at Taipei City Psychiatric Center, Taiwan. All participants were Hans in ethnicity and other minorities were excluded from this study. Excluded were also those who were on a low fat diet or vegetarians. The healthy controls were free from having any positive family history of mental disorder and taking any psychotropic agents.

Enrolled patients met DSM-IV (American Psychiatric Association, 1994) criteria for bipolar I disorder, most recent episode manic. The bipolar patients who had mixed episode of mood symptoms or other comorbid Axis I psychiatric disorders (i.e., psychiatric disorders due to a general medical condition or induced by substance uses) were excluded. During the index hospitalization, all patient subjects continued to receive existing mood stabilizers, benzodiazepine and antipsychotic drugs. After having signed written informed consent, the patients and controls were sampled for venous blood between 08:30 and 09:30 after overnight fasting.

Blood samples were analyzed for individual fatty acids with gas chromatography of methyl esters. Individual fatty acids were identified by comparison of gas chromatograms (Lipid Standards, FAMES, Sigma, St. Louis, MO, USA). The detailed step-by-step procedures are described elsewhere (Edwards et al., 1998; Maes et al., 1999). Briefly, 0.5 g of centrifuged, washed red blood cells was placed into 16×150 mm test tubes with Teflon-lined screw caps, followed by addition of 2 ml methanol–benzene solution (1:1, v/v). Samples were vortexed at low speed while slowly adding 200 μ l of acetyl chloride. Then, they were gassed with N_2 , capped tightly and heated at 100 °C for 30 min. After samples were cooled to room temperature, 5 ml of 6% K_2CO_3 was added and the sample vortexed for 30 s. Thereafter, samples were centrifuged 10 min at 1500×g, and the benzene layer (upper layer) was taken and washed three times (10 min at 1500×g) with distilled, deionized water. The upper layer was then removed and placed in injection vials for analysis. Heptadecaenoic acid was added as the internal standard. Fatty acid methyl esters (FAMES) were analyzed by capillary gas chromatography (Hewlett-Packard 5890 II Plus, Hewlett-Packard, Palo Alto, CA, USA) equipped with a 25 m×0.32 mm I.D. capillary column (Hewlett-Packard FFAP, 0.25 g film thickness, Hewlett-Packard) and flame ionization detection. The injector and detector temperatures were 230 and 250 °C, respectively, and the split ratio was 100:1. Initially, the oven temperature was set at 160 °C for 4 min, and was then increased at 2.5 °C/min to 225 °C and held for 20 min. Peaks were integrated by a programmable integrator (Hewlett-Packard 3395, Hewlett-Packard). Fatty acid profiles were identified by comparing the retention times with those of appropriate standard FAMES. Laboratory measures were conducted on coded samples by workers who were blind to the information whether samples were from bipolar subjects or controls.

Data were analyzed by using the Statistical Package for

Social Sciences, Version 9.0 (SPSS Inc.). Fatty acid profiles between bipolar patients and normal controls, medicated and drug-free patients, and smoker and nonsmoker patients were compared by independent *t*-test. Among bipolar patients in acute mania, Spearman's correlations were used to examine the relationship of continuous variables of the clinical features to each of PUFAs. Difference was considered statistically significant if a *P*-value was equal to or smaller than 0.05.

3. Results

The participating subjects included 20 (10 male and 10 female) manic patients with a mean age of 39.0 ± 10.5 (S.D.) years, and 20 (9 male and 11 female) healthy controls with mean age of 38.7 ± 12.8 (S.D.) years. The body mass index in patients and controls were 26.4 ± 8.2 (S.D.) and 23.2 ± 3.2 (S.D.), respectively. There was no significant difference in the distribution of age, gender and body mass index between these two groups. In bipolar patients, the age of onset was 26.5 ± 9.9 (S.D.) years with duration of disease being 11.1 ± 9.6 (S.D.) years. Numbers of mood (manic or depressive) episodes were 5.2 ± 4.5 (S.D.) and numbers of hospital admission were 3.8 ± 3.2 (S.D.). Further, the mean scores of Young Mania Rating Scale (Young et al., 1978) in patients with acute mania at the time of blood sampling for the study was 32.1 ± 8.8 (S.D.) in a range of 14–43.

Five patients at the time of blood sampling were free of any psychotropic drugs for at least 1 week and their blood levels of mood stabilizers were undetectable. However, 15 patients (75%) in this study were treated with mood stabilizers, including lithium ($n=9$), valproate ($n=5$), and valproate with carbamazepine ($n=1$). In addition, 10 of 15 those patients were also receiving antipsychotic drugs.

Table 1 shows erythrocyte membrane fatty acid levels in bipolar patients and controls. There was no difference found between patients and control subjects for total omega-3 PUFA and omega-6 PUFA levels. However, there is significant decreased concentration of AA and DHA in the patient group compared with the control group. The concentrations of another omega-3 or omega-6 fatty acids were not significantly different between patient and control groups. As to the ratio of AA and eicosapentaenoic acid and that of omega-6 PUFA and omega-3 PUFA, no statistical differences were found.

The medication effects on PUFA in bipolar patients were examined (data not shown). The 15 medicated patients had non-significantly different mean values \pm S.D. of AA (1.03 ± 0.50) from that of five drug-free patients (1.38 ± 0.95). The mean value \pm S.D. of DHA in medicated patients (0.65 ± 0.49) was also comparable to that of drug-free patients (1.33 ± 1.17). We also examined the possible influences of clinical variables on the change of PUFAs, including current age, age of onset of the disease, number of episodes and length of illness. DHA and AA com-

Table 1

Erythrocyte membrane fatty acid levels between bipolar manic and normal controls

RBC membrane ^a	Bipolar manic (<i>n</i> =20)	Normal controls (<i>n</i> =20)	<i>P</i> value
PUFA, n-3 (%)			
18:4	0.84 (0.59)	0.53 (0.57)	0.102
20:5 (EPA)	1.14 (1.66)	0.86 (0.58)	0.484
22:6 (DHA)	0.82 (0.75)	1.58 (0.68)	0.002 ^b
PUFA, n-6 (%)			
18:2	8.43 (2.83)	7.33 (2.57)	0.210
18:3	1.30 (0.73)	0.95 (0.92)	0.198
20:4 (AA)	1.12 (0.63)	2.39 (0.95)	0.000 ^b
Total n-3	4.09 (2.67)	3.92 (2.07)	0.820
Total n-6	9.54 (3.03)	9.72 (2.95)	0.851
Ratios			
AA/EPA	2.63 (2.84)	5.07 (5.52)	0.086
n-6/n-3	3.05 (1.59)	3.02 (1.63)	0.963

^a Erythrocyte values are presented as mg/100 mg of total phospholipid.

^b Significantly different ($P < 0.05$).

ponents of the erythrocyte membrane in acute mania were not found correlated to any aforementioned clinical variables. To evaluate the possible smoking effects on fatty acids, we divided the patient group into nonsmokers ($n=13$) and smokers ($n=7$). There was no difference in any of the PUFA compositions between the two groups. The mean values \pm S.D. of AA in smokers and nonsmokers were 1.13 ± 0.64 and 1.11 ± 0.66 ($P=0.962$), respectively. For DHA, the mean values \pm S.D. in smokers and nonsmokers were 0.51 ± 0.45 and 0.99 ± 0.83 ($P=0.178$), respectively.

4. Discussion

To the best of our knowledge, this is the first controlled study focusing on the PUFA levels of erythrocyte cell membranes in bipolar patients. The major finding of this study is that AA and DHA compositions in bipolar patients were significantly reduced as compared to normal controls.

The abnormalities in PUFA compositions may vary in different major psychiatric disorders. In the depressive patients, the major abnormality is a significant decrease in total omega-3 PUFAs, including EPA and DHA (Adams et al., 1996; Edwards et al., 1998; Maes et al., 1996, 1999; Peet et al., 1998). In contrast, the depletions of linoleic acid (18:2 ω -6), AA and DHA have been most often replicated in schizophrenic patients (Assies et al., 2001; Glen et al., 1994; Peet et al., 1995; Vaddadi et al., 1996). The results of our study revealed the reduced erythrocyte DHA levels in patients with manic episode of bipolar disorder. Omega-3 PUFAs, especially DHA, in the central nervous system are thought to have mood-stabilizing effects due to the action on serotonergic neurotransmission

(Delion et al., 1996), alteration in membrane “fluidity” (Bourre et al., 1993), as well as suppression of phosphatidylinositol (Medini et al., 1990) and protein kinase C (Seung Kim et al., 2001) signal transduction. Study data on primates (Neuringer et al., 1984) and human infants (Makrides et al., 1994) indicate a good correlation between erythrocyte and brain omega-3 PUFA compositions, and suggest that our patients’ reduced erythrocyte DHA level could reflect their deficit in the brain. Biochemical studies (Medini et al., 1990) further revealed that high dose treatment of omega-3 PUFAs incorporates these compounds into membrane phospholipids. Taken together, all this evidence provides a rationale to use omega-3 PUFAs to treat patients with bipolar disorder (Stoll et al., 1999), schizophrenia (Peet et al., 2001), and major depressive disorder (Nemets et al., 2002; Su et al., 2002 unpublished). It is interesting to note, EPA, but not DHA, is reported to improve symptoms of patients with schizophrenia (Peet et al., 2001) and major depressive disorder (Nemets et al., 2002). Stoll et al. (1999) suggested that the combination of DHA and EPA could exhibit mood-stabilizing properties in bipolar disorder. However, no difference in EPA level between manic patients and healthy controls was found in our study. Whether EPA is more effective than DHA as a mood stabilizer needs further investigation.

Both omega-6 and omega-3 PUFAs are potent modulators of the inflammatory response system (IRS). The omega-6 PUFAs, AA in particular, are precursors of proinflammatory eicosanoids and have been found positively related to the production of proinflammatory cytokines (Maes et al., 2000). Overactivation of AA metabolism induces depletion of erythrocyte AA concentration and IRS activation in patients with bipolar disorder (Maes et al., 1995; Su et al., 2002; Tsai et al., 1999, 2001). Chang et al. (2001) also found that lithium and valproic acid have a common action in reducing turnover of AA in rat brain. Those data are in agreement with the finding of significant decreased level of AA in our study.

There are some limitations in our study. The diet of the patients and controlled subjects in this study was not controlled and assessed due to the reliability problems as well as the psychopathology of patients in acute manic state. Katan et al. (1997) and Kinsella (1990) reported that erythrocyte membrane composition can be influenced by long-term dietary pattern. Further studies should be designed to control and to assess the diet when the patients are hospitalized. Although no significant difference in fatty acid compositions between medicated and drug-free patients was found, we still cannot exclude the possible effects of psychotropic drugs on PUFA levels because antipsychotic drugs are found to alter membrane’s phospholipid composition (Horrobin et al., 1997) and the mood stabilizers to reduce the AA turnover rate (Chang et al., 2001). Besides, the possible effect of smoking also needs to be addressed because smoking has been reported to deplete PUFAs from membranes, including nonspecific

degradation of not only DHA and docosapentaenoic acid from n-3, but also the PUFAs from n-6 series (Pawloski et al., 1999). The results in our study indicated the differences of fatty acid compositions between smoker and nonsmoker groups were not statistically significant.

In conclusion, our study showed that the depletion of AA and DHA of erythrocyte membrane in bipolar manic patients is significant as compared with that in healthy controls. This abnormality may be related to the mechanism of action of mood stabilizers and the previous findings on the abnormal psychoimmunology of patients with bipolar disorder. Possible mechanisms of having low membrane AA and DHA in bipolar disorder include reduced availability of PUFAs, reduced cellular uptake of PUFAs, deficient incorporation of PUFAs into membrane phospholipids, deficient activity of both enzyme (desaturase or elongase), or excessive removal by membrane lipid peroxidation (Mahadik et al., 1996). However, the possible mechanisms were not investigated in this study. Further investigations about the role and mechanisms of depleted AA and DHA or other PUFA related to these enzymes in bipolar disorder are warranted. A study on larger sample size of medicated patients or drug-free manic, well-controlled designs on the diet and smoking, and fatty acid composition measurements during full remission after the index episode are needed.

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