Serum Folate, Vitamin B_{12}, and Homocysteine in Major Depressive Disorder, Part 1: Predictors of Clinical Response in Fluoxetine-Resistant Depression

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Objective: In the present study, we assessed the relationship between serum folate, vitamin B_{12}, and homocysteine levels and clinical response in patients with major depressive disorder (MDD) who had previously failed to respond to open treatment with fluoxetine 20 mg/day and were enrolled in a 4-week, double-blind trial of either (1) fluoxetine dose increase, (2) lithium augmentation of fluoxetine, or (3) desipramine augmentation of fluoxetine.

Method: Fifty-five outpatients (mean ± SD age = 41.7 ± 10.6 years; 50.9% women) with MDD as assessed with the Structured Clinical Interview for DSM-III-R who were enrolled in the double-blind trial had serum folate, vitamin B_{12}, and homocysteine measurements completed at baseline (prior to fluoxetine treatment initiation). Folate levels were classified as either low (≤ 2.5 ng/mL) or normal. Vitamin B_{12} levels were classified as either low (≤ 200 pg/mL) or normal. Homocysteine levels were classified as either elevated (≥ 13.2 μmol/L) or normal. With the use of a logistic regression, we then assessed the relationship between (1) low or normal folate levels, (2) normal or low B_{12} levels, and (3) elevated or normal homocysteine levels and clinical response to double-blind treatment. The study was conducted from November 1992 to January 1999.

Results: Low serum folate levels ($\chi^2 = 3.626$, $p = .04$), but not elevated homocysteine ($p > .05$) or low vitamin B_{12} levels ($p > .05$), were associated with poorer response to treatment. The response rates for patients with (N = 14) and without (N = 38) low folate levels were 7.1% versus 44.7%, respectively.

Conclusion: Low serum folate levels were found to be associated with further treatment resistance among patients with fluoxetine-resistant MDD.

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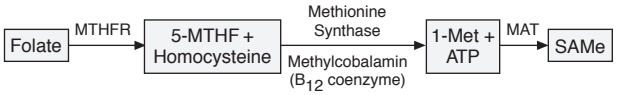
A number of studies suggest that many patients with major depressive disorder (MDD) also present with dysregulation in one-carbon metabolism,[1–3] which involves the production of *s*-adenosylmethionine (SAMe) from adenosine triphosphate and l-methionine[2] (Figure 1), although not all studies support the relationship between hypofolatemia and depression.[4] A number of these studies particularly focus on the presence of abnormally low serum or red blood cell folate (RBCF) concentrations in patients with MDD.[3,5] In turn, RBCF concentration has been reported to directly correlate with cerebrospinal fluid 5-hydroxyindoleacetic acid and homovanillic acid levels in MDD patients.[6] In addition, there are several reports of low or lower folate concentrations in MDD patients than in patients with other psychiatric disorders and healthy comparison subjects,[7–10] particularly for patients with melancholic depression.[11] In parallel, RBCF concentrations[12] and 5-methyltetrahydrofolate (5-MTHF) levels[13] have been found to inversely correlate with the severity of depression in MDD. Finally, a certain mutation *(C677T)* in the gene coding for the MTHF-reductase (MTHFR) enzyme, involved in the production of MTHFR,[14] was found to be more prevalent in patients with MDD than in controls[15] and to predict the onset of late-life depression.[16] Our group recently estimated the prevalence of the *C677T*

Figure 1. SAMe-Folate-Homocysteine-B_{12} Pathways[a]



[a]Adapted with permission from Mischoulon D, Rosenbaum J, eds. Natural Medications for Psychiatric Disorders: Considering the Alternatives. Philadelphia, Pa.: Lippincott Williams & Wilkins; 2002.
Abbreviations: ATP = adenosine triphosphate, MAT = methionine adenosyltransferase, 1-Met = methionine, 5-MTHF = 5-methyltetrahydrofolate, MTHFR = methyltetrahydrofolate reductase, SAMe = *s*-adenosylmethionine

allele of the MTHFR gene, associated with poorer conversion of folate to SAMe, to be approximately 58% (47% heterozygotes, 11% homozygotes).[17]

A number of studies also reveal the presence of low serum folate levels to have an adverse impact on the treatment of MDD.[11,12,18,19] Our group[11] previously reported that MDD patients with pretreatment serum folate levels less than or equal to 2.5 ng/mL were less likely to respond to an 8-week, fixed-dose, open trial of 20 mg of fluoxetine. Nonresponders to the 8-week open trial were subsequently treated in a double-blind fashion with either higher doses of fluoxetine, desipramine augmentation of fluoxetine, or lithium augmentation of fluoxetine for a total of 4 weeks. The purpose of the present study was to explore the potential relationship between one-carbon cycle metabolism abnormalities and antidepressant nonresponse to the double-blind phase of the study.

METHOD

Outpatients, aged 18–65 years, who met criteria for a current major depressive episode according to the Structured Clinical Interview for DSM-III-R, Patient Edition (SCID-P),[21] who were medication-free for at least 2 weeks and who had a baseline 17-item Hamilton Rating Scale for Depression (HAM-D-17)[22] score ≥ 16 were eligible to enroll in an 8-week, fixed-dose, open-label trial of 20 mg of fluoxetine conducted at the Massachusetts General Hospital Depression Clinical and Research Program, Boston, from November 1992 to January 1999. Patients were recruited through radio advertisements, newspaper advertisements, or colleague referrals.

Exclusion criteria included pregnant women; women of childbearing potential who were not using a medically accepted means of contraception; lactating women; patients with serious suicidal risk or serious, unstable medical illness; and patients with a history of seizure disorder. Patients with any of the following DSM-III-R diagnoses were also excluded: organic mental disorders; substance use disorders, including alcohol, active within the last year; schizophrenia; delusional disorder; psychotic disorders not elsewhere classified; bipolar disorder; or antisocial personality disorder. Further exclusion criteria included patients with a history of multiple adverse drug reactions or allergy to the study drugs; patients with mood congruent or mood incongruent psychotic features; patients currently using other psychotropic drugs; patients with clinical or laboratory evidence of hypothyroidism; patients whose depression had failed to respond in the past to a trial of either higher doses of fluoxetine (60–80 mg/day), the combination of fluoxetine and desipramine, or the combination of fluoxetine and lithium; and patients who had failed to respond during the course of their current major depressive episode to at least 1 adequate antidepressant trial, defined as 6 weeks or more of treatment with either > 150 mg of imipramine (or its tricyclic equivalent) or > 60 mg of phenelzine (or its monoamine oxidase inhibitor equivalent).

During the screen visit, all enrolled patients signed an Institutional Review Board–approved written informed consent form. A medical and psychiatric history, physical examination, serum chemistries, hematologic measures, electrocardiograph, and urine pregnancy test were then performed. The 31-item version of the Hamilton Rating Scale for Depression (HAM-D-31),[22] which allows the scoring of the HAM-D-17, was also administered during the screen visit and all subsequent visits. The screen visit was conducted by experienced psychologists or psychiatrists extensively trained in the use of the HAM-D-31 through didactic sessions and periodic reviews of videotaped interviews. Our interrater reliability for the use of the SCID-P was recently estimated as $\kappa = 0.80$.[23] At the conclusion of the screen visit, all enrolled patients were asked to return 1 week later for the baseline visit.

Patients returning for the baseline visit were then treated openly with fluoxetine 20 mg/day for 8 weeks, with visits every other week, after which they became eligible for a 4-week, double-blind, triple-dummy, randomized study if they continued to meet criteria for MDD and if they were treatment-resistant. Resistance to treatment was defined as a failure to achieve a 50% or greater reduction in the HAM-D-17 scores and a HAM-D-17 score > 10 at the time of randomization (end of the acute phase of treatment). There was a prospective stratification of the patients for the randomization based on whether they were partial responders or nonresponders: partial responders were those patients who had experienced a > 25% and < 50% reduction in their HAM-D-17 score or had a HAM-D-17 score < 16; nonresponders were those patients who had a < 25% reduction in their HAM-D-17 score from the beginning of treatment and had a HAM-D-17 score ≥ 16. All subjects were taking fluoxetine 20 mg/day when they entered the double-blind portion of the study.

A total of 386 outpatients volunteered to participate in the study and were treated openly with fluoxetine 20 mg/day. Of these 386 patients, 101 (26.2%) did not

respond adequately to the open fluoxetine treatment and agreed to be randomized to the double-blind phase. In the double-blind phase, subjects were randomly assigned to 1 of 3 different treatments: fluoxetine 40 to 60 mg/day, fluoxetine 20 mg plus desipramine 25 to 50 mg/day, or fluoxetine 20 mg plus lithium 300 to 600 mg/day.

Once enrolled in the double-blind phase, patients were seen weekly for a total of 4 weeks. The HAM-D-31 was administered during each of these visits. During the first week of the double-blind phase, all patients were instructed to take 1 capsule of fluoxetine 20 mg and 1 study capsule (fluoxetine or fluoxetine-placebo) every morning, and to take 1 tablet of desipramine 25 mg or desipramine-placebo and 1 tablet of lithium 300 mg or lithium-placebo at bedtime. After 1 week on this regimen, investigators were permitted either to increase the dosage if the patient was not responding or to maintain the same regimen. Doses were increased according to schedule until a remission of depressive symptoms occurred; however, side effects determined whether the dosage was maintained at the same level or reduced. In sum, patients were taking fluoxetine 40 to 60 mg/day, fluoxetine 20 mg plus desipramine 25 to 50 mg/day, or fluoxetine 20 mg plus lithium 300 to 600 mg/day.

Folate, Vitamin B_{12}, and Homocysteine Assays

Blood samples for folate, vitamin B_{12}, and homocysteine were collected during the baseline visit for the open trial. Since this was an add-on investigation, folate/vitamin B_{12}/homocysteine levels were only available in 55 of 101 patients. Once obtained, the serum was stored at –20ºC. The serum specimens were then blindly assayed for folate, vitamin B_{12}, and homocysteine in bulk at a later date. Although the duration of sample storage was variable, plasma folate levels have been shown to be stable when stored at –20ºC, and long-term stability over 4 years has been demonstrated.[24] Similarly, total plasma homocysteine has been shown to be extremely stable over 10 years when stored at –20ºC.[25] Since samples were assayed in bulk much later, patients and clinicians were blinded to folate, vitamin B_{12}, or homocysteine status during treatment.

Serum folate and B_{12} levels were determined by radioassay using purified intrinsic factor and purified folate-binding protein (Quantaphase, Bio-Rad Laboratories, Richmond, Calif.). Homocysteine levels were determined by high-pressure liquid chromatography with fluorescence detection following precolumn derivatization with 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole-4-sulfonate.[26] The coefficient of interassay variation was 4.2%, and the intra-assay variation was 6.8%.

In line with our previous study,[11] all metabolite levels were categorically defined. Specifically, the Bio-Rad reference ranges (Bio-Rad Laboratories, Richmond, Calif.) were used to classify the levels of folate (low: 1.5–2.5 ng/mL) and vitamin B_{12} (low: 160–200 pg/mL). We did not have a sufficiently large group of samples to define our own reference ranges and thus used the Bio-Rad reference ranges, which are based on the testing of samples taken from 238 apparently healthy adults undergoing routine physical examination, 105 B_{12}-deficient patients, and 46 folate-deficient patients. These reference ranges are widely used in clinical chemistry laboratories to diagnose folate and B_{12} deficiency. Because a reference range from a large database was unavailable for homocysteine, the homocysteine levels from the depressed cohort were compared with those of a smaller group of 48 comparison subjects with a mean age of 38.5 years (SD = 11.9), 20 (41.7%) of whom were women. Their mean homocysteine levels were 7.3 μmol/L (SD = 2.9). Depressed subjects whose homocysteine levels were 2 standard deviations above the comparison mean were classified as having elevated homocysteine levels (13.2–16.0 μmol/L). The homocysteine assay was developed in Dr. Bottiglieri's laboratory, and the comparison range we defined (from 48 healthy adult volunteers) is in good agreement with published reference ranges obtained in several other laboratories.[25] For demographic and laboratory variables, all values are mean ± SD unless otherwise stated.

Statistical Tests

Chi-square and t tests were used to compare patients in the double-blind trial who did and did not have folate, B_{12}, or homocysteine levels measured at baseline. A responder was defined as having a 50% or greater reduction in HAM-D-17 score from baseline (visit 1 of the adjunct study) to endpoint.[27] An intent-to-treat analysis was used to define the severity of depression at endpoint, in which the last recorded HAM-D-17 score substituted the score at week 4 for patients who prematurely discontinued the study. Chi-square and t tests were used to compare patients with low and normal serum folate levels with respect to age, gender, body mass index (BMI, in kg/m^2), and the severity of depression during the baseline visit for the second phase of the trial (4-week, double-blind, triple-dummy, randomized study) as reflected by the HAM-D-17 total score during that visit. This comparison was repeated for the samples with low and normal B_{12} levels and for the samples with elevated and normal homocysteine levels. A logistic regression was performed with folate level status, vitamin B_{12} level status (low vs. normal), homocysteine level status (elevated vs. normal), and baseline HAM-D-17 score as the independent variables and clinical response as the dependent variable. For all analyses, statistical significance was set at $p \leq .05$.

RESULTS

The results of the acute phase of the trial are reported elsewhere.[28] Of the 101 patients in the double-blind phase, 55 patients had serum folate, B_{12}, and/or homo-

cysteine levels measured at baseline. There was no statistically significant difference between the patients enrolled in the double-blind trial who did (N = 55) and did not (N = 46) have folate, B_{12}, or homocysteine levels measured at baseline in gender ratio (28/55 women vs. 49/46 women, $p > .05$), age (41.7 ± 10.6 vs. 41.4 ± 10.8 years, $p > .05$), or the severity of depression during the baseline visit of the double-blind trial as reflected by the HAM-D-17 total score (16.8 ± 4.6 vs. 16.3 ± 4.4, $p > .05$). Of the 55 patients, 22 received lithium augmentation of fluoxetine, 18 received higher doses of fluoxetine, and 15 received desipramine augmentation of fluoxetine.

Fifty-two patients had serum folate levels measured at baseline. Of these, 14 (26.9%) had low folate levels (3 were folate deficient; < 1.5 ng/mL) while 38 (73.1%) had levels within normal limits. The mean folate level for our sample was 5.7 ± 4.9 ng/mL. There was no statistically significant difference with respect to age in years (37.7 ± 9.8 vs. 43.8 ± 10.8), gender ratio (42.8% vs. 50.0% women), BMI in kg/m^2 (25.3 ± 6.2 vs. 25.6 ± 4.6), or HAM-D-17 score at baseline (17.8 ± 5.4 vs. 15.6 ± 3.8) between the groups with low and normal folate levels (all comparisons $p > .05$). Five patients with low serum folate underwent desipramine augmentation, 4 underwent lithium augmentation, and 5 received higher doses of fluoxetine ($p > .05$).

Fifty-five patients had serum vitamin B_{12} levels measured at baseline. Of these, 6 (10.9%) had low levels and 49 (89.1%) had levels within normal limits. The mean vitamin B_{12} level for the entire sample was 310.5 ± 124.6 pg/mL. Of the 6 patients with low B_{12} levels, 1 responded (16.7%), while of the 49 patients with normal B_{12} levels, 19 (38.8%) responded ($p < .03$). There was no statistically significant difference with respect to age (41.0 ± 10.9 vs. 41.8 ± 10.8 years), gender ratio (50.0% vs. 48.9% women), BMI in kg/m^2 (27.7 ± 6.9 vs. 25.5 ± 4.7), or HAM-D-17 score at baseline (15.0 ± 3.4 vs. 16.1 ± 4.4) between the groups with low and normal serum B_{12} levels (all comparisons $p > .05$). One patient with low vitamin B_{12} underwent desipramine augmentation, 1 underwent lithium augmentation, and 4 received higher doses of fluoxetine ($p > .05$).

Fifty-five patients had serum homocysteine levels measured at baseline. Of these, 15 (27.3%) had elevated homocysteine levels while 40 (72.7%) had levels within normal limits. The mean homocysteine level for our sample was 11.0 ± 4.1 μmol/L. Patients with elevated homocysteine levels were more likely to be older than patients with normal homocysteine levels (28.3 ± 6.4 vs. 25.1 ± 4.4 years, $p = .003$). There was no statistically significant difference with respect to gender ratio, BMI in kg/m^2 (28.3 ± 6.4 vs. 25.1 ± 4.4), or HAM-D-17 score at baseline (15.8 ± 5.1 vs. 16.0 ± 4.1) between patients with elevated and normal serum homocysteine levels (all comparisons $p > .05$). Three patients with elevated homocysteine underwent desipramine augmentation, 6 underwent lithium augmentation, and 6 received higher doses of fluoxetine ($p > .05$).

Low folate levels ($\chi^2 = 3.626$, $p = .04$), but not low vitamin B_{12} ($p > .05$) or elevated homocysteine levels ($p > .05$), were associated with an increased risk of nonresponse. Of the 14 patients with low folate levels, only 1 (7.1%) responded, while of 38 patients with normal folate levels, 17 (44.7%) responded.

DISCUSSION

The results of the present study reveal a significant relationship between serum folate or homocysteine level status at baseline and clinical response in fluoxetine nonresponders randomized to receive either higher doses of fluoxetine or augmentation of fluoxetine with lithium or desipramine. Specifically, the presence of hypofolatemia was found to confer a poorer response to double-blind treatment in patients with MDD. In fact, while approximately 44.7% of patients with normal serum folate levels responded to treatment, only 7.1% of patients with low folate levels responded. However, similar to our previous work,[11] there was no significant relationship between vitamin B_{12} or homocysteine levels and response to fluoxetine treatment.

The present results with regard to the association between hypofolatemia and poorer clinical response is in accordance with a growing number of studies. In addition to fluoxetine,[11] MDD patients with low folate levels also appear to have poorer response to treatment with sertraline,[18] desipramine,[12] and thyroid hormone[19] augmentation of antidepressants. In fact, a greater degree of change in RBCF concentrations during treatment with desipramine predicted a greater decrease in HAM-D scores during treatment, whereas greater RBCF concentrations after treatment were observed in responders than in nonresponders.[12] The correlation between the degree of increase of RBCF concentrations during treatment and the degree of change in HAM-D scores and clinical response was also replicated in a double-blind, placebo-controlled trial of thyroid hormone augmentation of antidepressants for MDD patients.[19] Finally, depressed adults treated with electroconvulsive therapy, antidepressants, or tryptophan who had lower pretreatment serum folate levels were also found to have higher depression and neuroticism ratings after treatment than patients with folate levels within normal limits.[20]

The present finding of a correlation between low folate levels and poorer response to "next-step" treatments in fluoxetine nonresponders implies that folate or SAMe supplementation may enhance response to "next-step" treatments among individuals who remain symptomatic following acute treatment with fluoxetine. SAMe supplementation may be especially pertinent for patients with

MDD who are carriers of the *C677T* allele of the MTHFR gene, associated with poorer conversion of folate to SAMe. While there have been no studies on this precise question, Coppen and Bailey[29] reported a more than 30% greater initial response rate to fluoxetine, at least in women, when folic acid (500 μg) versus placebo was added to their selective serotonin reuptake inhibitor (SSRI) regimen. Among subjects with SSRI-refractory depression, our group recently reported that folinic acid, a form of folate that enters the central nervous system as biologically active 5-MTHF, was associated with improved response in a proportion of subjects when added to the SSRI in an open trial.[30] Further exploration of the role of various folates and SAMe in optimizing antidepressant response to initial and subsequent treatment is warranted.

Limitations

One limitation of the present study is the relatively small sample size, which precludes any conclusions regarding relevance of low serum folate levels to response to any 1 of the 3 possible next-step treatments individually. Another limitation is that of sampling bias. Clinical trials have a number of inclusion and exclusion criteria and, as a result, patients in clinical trials do not directly reflect the typical outpatient population. The degree to which these findings generalize to a more heterogeneous population of depressed patients, including those with severe suicidality, psychosis, bipolar disorder, or substance abuse or those patients who are pregnant or who recently underwent childbirth, remains to be determined. The issue of generalizability may be particularly relevant in the case of pregnancy, recent childbirth, and alcoholism, all of which carry an increased risk of hypofolatemia.

In addition, whether patients were recruited through advertisements or referred by clinicians was not recorded in this study. Therefore, it is unknown whether there were any significant differences in folate levels between these 2 groups and whether any such differences contributed to outcome. Furthermore, interrater reliability was available for the SCID but not for the HAM-D-17. Also, in this study we measured serum folate. However, red blood cell folate may provide a more stable reflection of folate status and would be worthwhile to include in future studies. Finally, we did not repeat folate measures immediately after the conclusion of the open-label trial, which would allow us to assess whether changes in serum folate levels influenced the likelihood of clinical response.

CONCLUSION

The results of the present study reveal a significant relationship between serum folate levels and antidepressant response in patients with fluoxetine-resistant MDD treated with higher doses of fluoxetine, lithium augmentation of fluoxetine, or desipramine augmentation of fluoxetine. Specifically, the presence of hypofolatemia was associated with poorer response to double-blind treatment, with striking differences in response rates.

Part 2 of this 2-part series appears in this issue on pages 1096–1098.

Drug names: desipramine (Norpramin and others), fluoxetine (Prozac and others), imipramine (Tofranil and others), lithium (Lithobid, Eskalith, and others), phenelzine (Nardil), sertraline (Zoloft).

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