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ARTICLE

The relationship between serum folate, vitamin B12, and homocysteine levels in major depressive disorder and the timing of improvement with fluoxetine

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Abstract

The objective of the present study was to examine the relationship between serum folate, vitamin B12, and homocysteine levels and the timing of clinical improvement to fluoxetine in major depressive disorder (MDD) patients. A total of 110 outpatients with MDD who responded to an 8-wk trial of fluoxetine had serum folate, B12, and homocysteine measurements at baseline (prior to fluoxetine initiation). Onset of clinical improvement was defined as a 30% decrease in Hamilton Depression Scale scores that led to a 50% decrease by week 8. Patients with low folate levels ($\leqslant$2.5 ng/ml) were more likely to experience a later onset of clinical improvement than eufolatemic patients ($p=0.0028$). B12 and homocysteine level status did not predict time to clinical improvement ($p>0.05$). In conclusion, low serum folate levels were found to be associated with a delayed onset of clinical improvement during treatment with fluoxetine in MDD by, on average, 1.5 wk.

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Key words: Fluoxetine, folate, homocysteine, onset, response, vitamin B12.

Introduction

Folate and homocysteine are related through the one-carbon cycle, which involves the production of S-adenosyl methionine (SAMe) from adenosine triphosphate and l-methionine, the latter produced by the methylation of homocysteine by 5-methyltetrahydrofolate (5-MTHF) (Bottiglieri and Hyland, 1994). SAMe, which is uniformly distributed in the brain, serves as the major donor of methyl groups required in the synthesis of neuronal messengers and membranes (Bottiglieri and Hyland, 1994). Over the past few decades, an ever-increasing number of studies suggest that many patients with major depressive disorder (MDD) also present with dysregulation in one-carbon metabolism (Bottiglieri and Hyland, 1994), with the majority of these studies focusing on the presence of abnormally low serum or red blood cell folate (RBCF) concentrations in patients with MDD (Alpert and Fava, 1997; Papakostas et al., 2003) or lower serum/cerebrospinal fluid (CSF) SAMe levels (Bottiglieri et al., 1988, 1990). For example, in one study of patients with MDD, the RBCF concentration was found to directly correlate with CSF 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) levels (Bottiglieri et al., 1992) while, in a second study, Bottiglieri et al. (1990) reported that CSF SAMe levels were significantly lower in severely depressed patients than in a neurological control group. Furthermore, a number of studies also report patients with MDD to have low or lower folate concentrations more commonly than patients with other psychiatric disorders or normal comparison subjects (Abou-Saleh and Coppen, 1989; Carney and Sheffield, 1978; Ghadirian

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et al., 1980; Lee et al., 1998). In parallel, a number of studies also reveal the presence of low folate levels to have an adverse impact on the treatment of MDD (Alpert et al., 2003; Levitt et al., 1998; Reynolds et al., 1970). In a previous study conducted by our group, we found that MDD patients with pre-treatment serum folate levels $\leqslant$2.5 ng/ml were less likely to respond to an 8-wk, fixed-dose, open-trial of 20 mg fluoxetine (Fava et al., 1997). However, while a number of studies confirm a link between hypofolatemia and the subsequent risk of not responding to treatment, there is a paucity of studies exploring whether the presence of hypofolatemia confers poorer prognosis for treatment responders. For example, in a previous study, we found that hypofolatemia conferred an increased risk of relapse among fluoxetine remitters (Papakostas et al., 2004). Similar studies exploring the relationship between hypofolatemia and outcome for treatment responders would further strengthen the link between hypofolatemia and poorer outcome in MDD. Therefore, the purpose of this report was to study the relationship between folate, B12, and homocysteine and time to clinical improvement among fluoxetine responders from our previous trial (Fava et al., 1997).

Methods

Outpatients, aged 18–65 yr, who met criteria for a current major depressive episode according to the *Structured Clinical Interview for DSM-III-R* (SCID-P; Spitzer et al., 1989), who were medication-free for at least 2 wk, with a baseline 17-item Hamilton Depression Rating Scale (HAMD-17; Hamilton, 1960) score of $\geqslant$16 were eligible to enrol in an 8-wk, fixed-dose, open-label trial of 20 mg fluoxetine conducted at the Massachusetts General Hospital (MGH) Depression Clinical and Research Program (DCRP). Patients were recruited through radio advertisements, newspaper advertisements or colleague referrals.

Exclusion criteria included pregnant women and women of childbearing potential who were not using a medically accepted means of contraception, women of childbearing potential taking a birth control pill, lactating women, patients with serious suicidal risk or serious, unstable medical illness, patients with a history of seizure disorder, patients with the DSM-III-R diagnoses of 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, patients with a history of multiple adverse drug reactions or allergy to the study drugs, patients with mood-congruent or mood-incongruent psychotic features, current use of 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/d), or to the combination of fluoxetine and desipramine, or the combination of fluoxetine and lithium, patients who had failed to respond during the course of their current major depressive episode to at least one adequate antidepressant trial, defined as 6 wk or more of treatment with either $>$150 mg imipramine (or its tricyclic equivalent) or $>$60 mg phenelzine (or its monoamine oxidase inhibitor equivalent).

During the screen visit, all enrolled patients signed an Institutional Review Board (IRB)-approved written informed consent form. A medical and psychiatric history, physical examination, serum chemistries, haematological measures, electrocardiogram (EKG), and urine pregnancy test were then performed. The 31-item of the Hamilton Rating Scale for Depression (HAMD-31) was also administered during the screen visit. The screen visit was conducted by experienced psychologists or psychiatrists. In our group, training in the use of instruments such as the HAMD-31 and SCID-P is done by peer review of videotaped interviews. At the conclusion of the screen visit, all enrolled patients were asked to return 1 wk later for the baseline visit. Patients who returned for their baseline visit were started on a 20 mg, fixed-dose regimen of fluoxetine. Visits subsequent to the screen occurred at baseline and then every other week for a total 8 wk. The HAMD-31 was administered during all study visits.

Folate, vitamin B12, and homocysteine assays

The 213 subjects consecutively enrolled between between October 1992 and March 1995 also had blood samples for folate, vitamin B12, and homocysteine collected during their baseline visit (the original study protocol and budget did not provide for further enrolment). Once obtained, the serum was stored at $-20\,^{\circ}$C. In April 1995, the frozen serum specimens were blindly assayed for folate, vitamin B12, and homocysteine, the serum specimens were then blindly assayed for folate, vitamin B12, and homocysteine in bulk. Although the duration of sample storage was variable, plasma folate levels have been shown to be stable when stored at $-20\,^{\circ}$C, and long-term stability over 4 yr has been demonstrated (Kirke et al., 1993). Similarly, total plasma homocysteine has been shown to be extremely stable over 10 yr when stored at $-20\,^{\circ}$C (Ueland et al., 1993). Serum folate and B12

levels were determined by radioassay using purified intrinsic factor and purified folate-binding protein (Quantaphase; Bio-Rad Laboratories, Richmond, CA, USA). Homocysteine levels were determined by high-pressure liquid chromatography with fluorescence detection following pre-column derivatization with 4-(aminosulphonyl)-7-fluoro-2,1,3-benzoxadiazole-4-sulphonate (Ubbink et al., 1991). The coefficient of inter-assay variation was 4.2%, and the intra-assay variation was 6.8%.

Definition of outcome measures

Treatment response was defined as a 50% decrease in score on the HAMD-17 total score from baseline to end-point. A completer analysis was used to define end-point severity. Time to clinical improvement was defined as the first time-point at which the score on the HAMD-17 decreased by 30% from baseline without a subsequent increase that led to a 50% decrease by week 8 (Nierenberg et al., 2000). By including only those without any increase in HAMD-17 scores, we excluded patients who had a placebo pattern of non-sustained response (Quitkin et al., 1991). The responding group represented the best-case scenario: group members had a true drug pattern of response and responded or experienced remission by the end of the 8-wk trial. The rationale for segregating responders was that if non-responders were included in the same group, the time until onset of response would be delayed because of a reduced overall esponse rate and would lead to a false conclusion about the time until response for responders (Laska and Siegel, 1995).

Definition of folate, B12, and homocysteine level status

In line with our previous study (Fava et al., 1997) all metabolite levels were categorically defined. Specifically, the Bio-Rad reference ranges (Bio-Rad Laboratories) were used to classify the levels of folate (low: 1.5–2.5 ng/ml) and vitamin B12 (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 vitamin B12-deficient patients, and 46 folate-deficient patients. These reference ranges are widely used in clinical chemistry laboratories to diagnose folate and B12 deficiency. Because a reference range from a large database was unavailable for homocysteine, the homocysteine levels from the depressed cohort were compared to those of a smaller group of 48 comparison subjects, who had a mean age of 38.5 yr (S.D. = 11.9) and 20 (42%) of whom were female. Their mean homocysteine level was 7.3 μmol/l (S.D. = 2.9). Depressed subjects whose homocysteine levels were 2 S.D. 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 (Kirke et al., 1993).

Statistical analysis

χ^2 and t tests were used to compare sustained responders who did and did not have either folate, B12 or homocysteine levels at baseline with respect to age, gender, time to onset of clinical response and time to clinical response. χ^2 and t tests were used to compare responders with low and normal folate levels with respect to age, gender, body mass index (BMI; kg/m^2) and the severity of depression during the baseline visit. This comparison was repeated (1) for the samples with low and normal B12 levels and (2) for the samples with elevated and normal homocysteine levels. With the use of three separate analysis of covariance (ANCOVA) test and controlling for the severity of depression at baseline we then assessed the relationship between: (1) low or normal folate levels, (2) low or normal B12 levels, and (3) elevated or normal homocysteine levels and the time to onset of clinical improvement. For all analyses, statistical significance was set at $p \leqslant 0.05$.

Results

With regards to the overall timing of onset of clinical improvement, the results are reported elsewhere (Nierenberg et al., 2000). In summary, a total of 324 (84.4%) of the 384 patients in the study completed the open trial; there were 60 drop-outs (15.6%). Of the 384 patients, 193 (50.3%) responded, and 148 (38.5%) had acute remission with final HAMD scores of $\leqslant 7$. Of 324 who completed the study, 193 (59.6%) responded, and 148 (45.7%) experienced remission of their symptoms. A total of 182 (94.3%) of the 193 patients who met the criteria for response were included in the responder group; the criteria were (1) all data-points present, (2) a 30% decrease in baseline score on the HAMD without subsequent exacerbation during the acute phase, and (3) a 50% reduction in baseline score on the HAMD after 8 wk of treatment with 20 mg/d

fluoxetine. The remaining 11 patients were excluded from the responder group due to missing data and/or subsequent worsening following the initial improvement.

Of the 182 patients in the responder group, 108 had folate levels measured at baseline while 110 had homocysteine and B12 levels measured at baseline. There was no statistically significant difference between responders who did ($n=110$) and did not ($n=72$) have either folate, B12 or homocysteine levels at baseline in gender ratio (60/110 or 54.5% vs. 38/72 or 52.8% female, $p>0.05$) age (40.2 ± 10.3 vs. 41.1 ± 8.8, $p>0.05$), or the severity of depression during the baseline visit of the as reflected by the HAMD-17 total score (18.9 ± 2.7 vs. 19.4 ± 3.3, $p>0.05$). Patients who did ($n=110$) and did not ($n=72$) have either folate, B12 or homocysteine levels at baseline also did not differ in terms of the time to onset of response (3.6 ± 1.9 vs. 4.0 ± 2.0 wk, $p>0.05$), or the time to response (4.8 ± 2.0 vs. 5.0 ± 1.9 wk, $p>0.05$).

In total, 108 responders had folate levels measured at baseline. Of these, 16 (14.8%) had low folate levels while 92 (85.2%) had normal levels. The mean folate level for our sample was 6.4 ± 5.3 ng/ml. There was no statistically significant difference with respect to age in years (39.0 ± 9.0 vs. 40.3 ± 10.6 yr), gender ratio (9/16 or 56.2% female vs. 49/92 or 53.2% female), BMI (25.1 ± 7.2 vs. 25.7 ± 4.6 kg/m²) or HAMD-17 score at baseline (19.0 ± 2.7 vs. 18.6 ± 2.6) between the groups with low and normal folate (all comparisons $p>0.05$). The presence of low folate levels significantly predicted a later time to clinical improvement ($p=0.0028$; mean difference 1.5). The mean time to onset of clinical improvement for patients with low folate levels was 5.0 ± 1.8 vs. 3.5 ± 1.9 wk for patients with normal folate levels (see Figure 1 for more details).

A total of 110 responders had vitamin B12 levels measured at baseline. Of these, 15 (13.6%) had low levels and 95 (86.4%) had normal levels. The mean vitamin B12 level for the entire sample was 364.9 ± 173.4 pg/ml. There was no statistically significant difference with respect to age (37.6 ± 10.2 vs. 40.6 ± 10.3 yr), gender ratio (10/15 or 75.0% female vs. 50/95 or 52.6% female), BMI (28.1 ± 7.9 vs. 25.4 ± 4.5 kg/m²) or HAMD-17 score at baseline (19.2 ± 2.7 vs. 18.9 ± 2.7) between the groups with low and normal B12 levels (all comparisons $p>0.05$). Vitamin B12 level status did not predict time to onset of clinical improvement ($p=0.4$).

In total, 110 patients had homocysteine levels measured at baseline. Of these, 21 (19.1%) had elevated homocysteine levels while 89 (80.9%) had normal levels. The mean homocysteine level for our sample was 9.8 ± 4.1 μmol/l. There was no statistically significant difference with respect to age (40.9 ± 9.0 vs. 40.0 ± 10.6 yr), gender ratio (13/21 or 61.9% female vs. 47/89 or 52.8% female), BMI (28.3 ± 6.4 vs. 25.1 ± 4.4 kg/m²) or HAMD-17 score at baseline (19.0 ± 2.2 vs. 18.9 ± 2.8) between patients with elevated and normal homocysteine levels (all comparisons $p>0.05$). Hyperhomocysteinaemic patients presented with a higher BMI than non-hyperhomocysteinaemic patients (28.6 ± 7.7 vs. 25.1 ± 4.1 kg/m², $p=0.0055$) Homocysteine levels level status did not predict timing to onset of clinical improvement ($p=0.6$).

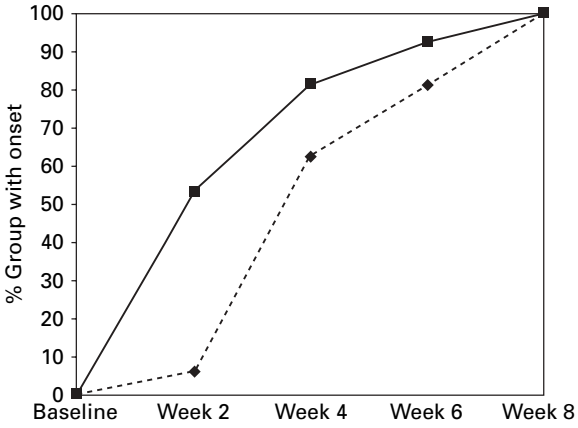

Figure 1. Proportion of group with onset of response per visit. --◆--, Low folate; —■—, normal folate.

Discussion

The results of the present study reveal a significant relationship between serum folate level and the time to clinical improvement during treatment with fluoxetine. Specifically, MDD patients with hypofolatemia treated with fluoxetine were more likely to experience a later time to onset of clinical improvement than eufolatemic patients by, on average, 1.5 wk, and this was regardless of depression severity at baseline. There was no significant relationship between vitamin B12 or homocysteine level status and the time to onset of clinical improvement.

A possible implication of the present finding between low folate and slower onset of clinical improvement with fluoxetine in MDD is that supplementation with either folate, or metabolic end-products of folate such as SAMe, could perhaps hasten the onset of response when co-administered during the acute phase of treatment with fluoxetine or other SSRIs. Coppen and Bailey (2000), for instance, studied

127 MDD outpatients who were co-administered to receive 20 mg fluoxetine daily in addition to 500 mg folic acid or placebo in a double-blind manner and found a significantly greater improvement in depressive scores in the folate group than the placebo group, suggesting that folate improved the antidepressant action of fluoxetine. While there have been no studies on this precise question with regards to combining SAMe with SSRIs, in a 2-wk, double-blind, placebo-controlled study of SAMe augmentation of imipramine in 40 MDD patients Berlanga et al. (2001) reported that depressive symptoms decreased earlier by the end of week 2 in the SAMe group than the placebo group. In parallel, our group had reported that 55% of 195 MDD patients treated with parenteral SAMe responded by day 15 of treatment, while a statistically significant drop in HAMD scores from baseline was noted even by day 7 (Fava et al., 1995). Therefore, in light of these preliminary findings, further exploration of the potential role of folate and its various metabolites such as SAMe in optimizing antidepressant response to initial and subsequent treatment is warranted.

Limitations

One limitation of the present study is that follow-up visits were performed every other week rather than every week as more frequent visits may have improved our ability to measure the timing of clinical improvement. In addition, the HAMD may have been insufficiently sensitive to measure the changes in depression associated with the time until onset of response. Furthermore, treatment was open, without blinding of the subjects or evaluators and no placebo group was included. 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 remains to be determined. In addition, in order to accurately estimate time to onset of response we have limited the present analysis to completers as in a previous report (Nierenberg et al., 2000). Thus, the degree to which these findings generalize to patients who discontinued the study also remains to be determined. Finally, in this study we measured serum folate. However, RBCF may provide a more stable reflection of folate status and would be worthwhile including in future studies.

Conclusion

The results of the present study reveal a significant relationship between serum folate level status at baseline and the time to clinical improvement with fluoxetine. Specifically, MDD patients with hypofolatemia were more likely to experience a later onset of clinical improvement than eufolatemic patients regardless of depression severity at baseline. Therefore, further exploration of the role of folate and its various metabolites such as SAMe in optimizing antidepressant response to initial and subsequent treatment is warranted.

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Statement of Interest

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References

Abou-Saleh MT, Coppen A (1989). Serum and red blood cell folate in depression. *Acta Psychiatrica Scandinavica 80*, 78–82.

Alpert JE, Fava M (1997). Nutrition and depression: the role of folate. *Nutrition Reviews 55*, 145–149.

Alpert M, Silva RR, Pouget ER (2003). Prediction of treatment response in geriatric depression from baseline folate level: interaction with an SSRI or a tricyclic antidepressant. *Journal of Clinical Psychopharmacology 23*, 309–313.

Berlanga C, Ortega-Soto HA, Ontiveros M, Senties H (2001). Efficacy of S-adenosyl-L-methionine in speeding the onset of action of imipramine. *Psychiatry Research 44*, 257–262.

Bottiglieri T, Chary TK, Laundy M, Carney MW, Godfrey P, Toone BK, Reynolds EH (1988). Transmethylation in depression. *Alabama Journal of Medical Sciences 25*, 296–301.

Bottiglieri T, Godfrey P, Flynn T, Carney MW, Toone BK, Reynolds EH (1990). Cerebrospinal fluid S-adenosylmethionine in depression and dementia: effects of treatment with parenteral and oral S-adenosylmethionine. *Journal of Neurology, Neurosurgery, and Psychiatry 53*, 1096–1098.

Bottiglieri T, Hyland K (1994). S-adenosylmethionine in psychiatric and neurological disorders: a review. *Acta Neurologica Scandinavica* (Suppl.) *154*, 19–26.

Bottiglieri T, Hyland K, Laundy M (1992). Folate deficiency, biopterin and monoamine metabolism in depression. *Psychological Medicine 22*, 871–876.

Carney MW, Sheffield BF (1978). Serum folic acid and B12 in 272 psychiatric in-patients. *Psychological Medicine 8*, 139–144.

Coppen A, Bailey J (2000). Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. *Journal of Affective Disorders 60*, 121–130.

Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T (1997). Folate, B12, and homocysteine in major depressive disorder. *American Journal of Psychiatry 154*, 426–428.

Fava M, Gianelli A, Rapisarda V, Patralia A, Guaraldi GP (1995). Rapidity of onset of the antidepressant effect of parenteral S-adenosyl-L-methionine. *Psychiatry Research 56*, 295–297.

Ghadirian AM, Anath J, Engelsman F (1980). Folic acid deficiency in depression. *Psychosomatics 21*, 926–929.

Hamilton M (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery, and Psychiatry 23*, 56–62.

Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM (1993). Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Quarterly Journal of Medicine 86*, 703–708.

Laska EM, Siegel C (1995). Characterizing onset in psychopharmacological clinical trials. *Psychopharmacological Bulletin 31*, 29–35.

Lee S, Wing YK, Fong S (1998). A controlled study of folate levels in Chinese inpatients with major depression in Hong Kong. *Journal of Affective Disorders 49*, 73–77.

Levitt AJ, Wesson VA, Joffe RT (1998). Impact of suppression of thyroxine on folate status during acute antidepressant therapy. *Psychiatry Research 79*, 123–129.

Nierenberg AA, Faragaugh AH, Altert JE, Gordon J, Worthington JJ, Rosenbaum JF, Fava M (2000). Timing of onset of antidepressant response with fluoxetine treatment. *American Journal of Psychiatry 157*, 1423–1428.

Papakostas GI, Alpert JE, Fava M (2003). SAMe in the treatment of depression: a comprehensive review of the literature. *Current Psychiatry Reports 5*, 460–466.

Papakostas GI, Petersen T, Mischoulon D, Green CH, Nierenberg AA, Bottiglieri T, Rosenbaum JF, Alpert JE, Fava M (2004). Serum folate, vitamin B12, and homocysteine in major depressive disorder, Part 2: predictors of relapse during the continuation phase of pharmacotherapy. *Journal of Clinical Psychiatry 65*, 1096–1098.

Quitkin FM, Rabkin JG, Stewart JW, McGrath PJ, Harrison W, Ross DC, Tricamo E, Fleiss J, Markowitz J, Klein DF (1991). Heterogeneity of clinical response during placebo treatment. *American Journal of Psychiatry 148*, 193–196.

Reynolds EH, Preece JM, Bailey J, Coppen A (1970). Folate deficiency in depressive illness. *British Journal of Psychiatry 117*, 287–292.

Spitzer RL, Williams JBW, Gibbon M, First M (1989). *Structured Clinical Interview for DSM-III-R – Patient edition* (SCID-P). New York: New York State Psychiatric Institute, Biometrics Research Department.

Ubbink JB, Vermark WJH, Bissbort S (1991). Rapid high-performance liquid chromatography assay for total homocysteine levels in human serum. *Journal of Chromatography 565*, 441–446.

Ueland PM, Refsum H, Stabler SP, Malinow RM, Andersson A, Allen RH (1993). Total homocysteine in plasma or serum: methods and clinical applications. *Clinical Chemistry 39*, 1764–1779.