THE EFFECT OF FOLIC ACID SUPPLEMENTATION ON THE TOXICITY OF LOW-DOSE METHOTREXATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Thirty-two patients with rheumatoid arthritis completed a 24-week, placebo-controlled, double-blind trial of folic acid (FA) supplementation during low-dose methotrexate (MTX) therapy. Administration of the daily FA supplement significantly lowered toxicity scores without affecting efficacy, as measured by joint counts, joint indices, and patient and physician evaluation of disease activity. Fifteen patients experienced some sort of toxicity; 67% were in the placebo group, and 33% were in the FA supplement group. Four patients in the placebo group had toxicity levels serious enough to require discontinuation of the MTX, while no patients in the FA supplement group discontinued MTX because of toxicity. Low-normal initial plasma and red blood cell folate levels were predictive of future toxicity with MTX therapy. We conclude that a daily supplement of 1 mg of FA during low-dose MTX therapy (median dose 7.5 mg/week [16.4 μmoles]) is useful in lessening toxicity without altering efficacy during the first 6 months of treatment.

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The use of the folic acid (FA) antagonist, aminopterin, for the treatment of rheumatoid arthritis (RA) was first reported by Gubner et al in 1951 (1). Numerous double-blind, placebo-controlled trials have since established the efficacy of *N*-10-methylaminopterin (methotrexate; MTX) in the treatment of RA (2–7). A meta-analysis of numerous studies has shown that patients receiving MTX for RA have a 26% greater improvement in their joint counts and a 39% greater improvement in pain scores than do control patients receiving nonsteroidal antiinflammatory drugs (NSAIDs) with or without prednisone. The major factor limiting MTX treatment seems to be its toxicity (9,10). Toxic manifestations, such as nausea, stomatitis, abnormal liver function, cytopenia, and pulmonary toxicity, have generally been reported in 30–60% of patients (7–10), and in 1 study, 90% of patients experienced toxicity (6).

The folate status of patients before and during MTX therapy has received little attention, even though some clinical manifestations of folate deficiency, such as cytopenia, anorexia, stomatitis, and gastrointestinal (GI) intolerance (11,12), are also observed as toxic reactions during low-dose MTX treatment for RA. It was therefore postulated that the administration of FA would be useful in reducing toxic manifestations that occur during long-term treatment with low-dose MTX for RA. We report herein the results of a double-blind, placebo-controlled study designed to test this hypothesis.

PATIENTS AND METHODS

Patients. Thirty-nine patients with RA that fulfilled the American Rheumatism Association 1958 criteria for

Table 1. Baseline clinical and demographic characteristics of the rheumatoid arthritis patients studied*

	Folate group (n = 16)	Placebo group (n = 16)
Age	52.0 ± 14.6	50.9 ± 13.5
Males/females	3/13	3/13
Previous use of folate-containing vitamins†	5	2
Years of disease	8.7 ± 5.5	15.3 ± 11.0‡
Rheumatoid factor positive	15	16
Initial serum folate, ng/ml	8.0 ± 5.0 (2.0–20.9)	8.5 ± 6.0 (1.3–23.1)
Initial RBC folate, ng/ml	355.9 ± 222.8 (101–539)	361.3 ± 193.4 (146–772)
Concurrent use of NSAIDs/aspirin	14	16
Concurrent use of prednisone	9	8
Anatomic stage§	3.6 ± 1.3	3.9 ± 1.1
Carpal:metacarpal ratio§	0.50 ± 0.03	0.51 ± 0.03

* Values are the mean ± SD or the number of patients. Numbers in parentheses are ranges. RBC = red blood cell; NSAIDs = nonsteroidal antiinflammatory drugs.
† Mean 400 μg folate/day.
‡ $P < 0.05$.
§ For explanation, see refs. 14–16.

definite or classic disease (13) gave informed consent and entered the study, which was approved by the Institutional Review Board of The University of Alabama at Birmingham. Seven patients could not be included in the data analysis: 4 because of noncompliance with the MTX regimen, 2 because of administration of intramuscular MTX, and 1 because of self-medication with large amounts of FA. None of these 7 patients were withdrawn because of the occurrence of toxic manifestations. Demographic and clinical characteristics of the 32 patients who completed the study are presented in Table 1.

Criteria for entry into the study included RA of more than 6 months duration, with onset after 16 years of age, and at least 3 of the following: ≥3 swollen joints, ≥6 or more tender joints, ≥45 minutes of morning stiffness, and a Westergren erythrocyte sedimentation rate (ESR) of ≥28 mm/hour. Radiographs were read by the project rheumatologist (GSA) in a blinded manner. The anatomic grading criteria of Berens and Lin (14), as modified by Trentham and Masi (15), as well as the carpal:metacarpal (C:MC) ratio (15), were used to assess the radiographs. These measurements had been previously used and validated at our institution and had been found to be reliable (16).

Patients with serious concomitant medical illnesses such as cancer, liver or renal disease, liver enzyme levels more than twice the upper limit of normal, white blood cell (WBC) counts <3,500/mm^3, or platelet counts <150,000/mm^3 were excluded from the trial. Previous use of MTX within the past 6 months and treatment with total lymphocyte irradiation were also exclusion criteria. Patients were not accepted into the trial until gold salts, D-penicillamine, sulfasalazine, or hydroxycloroquine treatment had been discontinued for at least 10 days.

During the study, each patient remained under the care of his or her rheumatologist, abstained from alcohol, and continued to receive stable doses of aspirin and/or NSAIDs. For those taking prednisone at study entry, the dosage was kept stable, not to exceed 10 mg/day. It was required that both male and female patients either be practicing contraception or have no reproductive potential.

Study design. The study was a double-blind, placebo-controlled trial of 24 weeks duration. Patients were assigned to receive folate (1 mg [2.2. μmoles]/day) or placebo. The 2 groups were matched, by the study statistician, on the basis of sex, previous use of folate-containing vitamins, rheumatoid factor (RF) serology, and age. Identical FA- and placebo-containing capsules were prepared by the Investigational Drug Service of the University of Alabama Hospital, using capsules provided by Capsugel (Warner Lambert, Greenwood, SC). Folic acid (pteroylglutamic acid) was obtained from Lederle Laboratories (Pearl River, NY). Spectrophotometric analysis indicated that the mean ± SD folate content was 1.03 ± 0.16 mg per capsule.

Folate-containing vitamin preparations, if previously used, were stopped for the duration of the study. Oral MTX was begun at a dosage of 2.5–7.5 mg per week, and was increased in 2.5-mg increments at the discretion of the treating rheumatologist. The weekly dosage of MTX did not exceed 15 mg, and the median dosage for both groups was 7.5 mg/week. The MTX tablets were generally ingested in equal numbers on 3 consecutive occasions at 12-hour intervals, always beginning on the same day of the week. Neither the investigators, the patients, nor the treating rheumatologists were aware of the placebo/folic acid capsule assignments until the study was completed. Patients were withdrawn from MTX therapy at the discretion of the treating rheumatologist.

Patients were evaluated immediately prior to initiation of MTX (visit 1), and after approximately 12 weeks (range 10–14 weeks; visit 2) and 24 weeks (range 22–26 weeks; visit 3) of MTX therapy. Patients were withdrawn from the study if they missed more than 3 weeks of MTX treatment for any reason.

Clinical assessment. Each patient was examined and interviewed by the same physician/nutritionist (SLM) and rheumatology research assistant (JVA or PKY). Medication compliance was determined by direct questioning, and by pill counts when possible.

The following clinical variables (5) were determined at each visit: 1) the number of joints with swelling (of 58 diarthrodial joints); 2) the number of joints with tenderness on pressure, pain on passive motion, or both (of 60 joints); 3) the joint swelling index, expressed as a sum, in which each joint was graded for swelling as 0 (none), 1 (mild), 2 (moderate), or 3 (severe); 4) the joint tenderness/pain index, expressed as a sum with joints also graded on the above scale of 0 (none) to 3 (severe); 5) mean grip strength for both hands; 6) the duration of morning stiffness, to the nearest hour; 7) the patient's assessment of disease activity, graded as 0 (asymptomatic), 1 (mild), 2 (moderate), 3 (severe), or 4 (very severe); 8) the physician's assessment of disease activity, graded on the same scale; 9) 1-day dietary recall,

using food models, which was coded using the Ohio State Nutrient Data Base (Unisoft Systems Associates, Columbus, OH); 10) current medications and dosages; and 11) toxic side effects. Before beginning MTX, each patient was counseled by his or her attending rheumatologist with regard to possible toxic side effects. The presence of such side effects was investigated by asking the question, "Is the MTX medication causing you any problems?" Toxicity was also noted and recorded every 3 weeks by telephone interview.

A toxicity score (TS) was determined for each patient. The scoring system was based on the clinical experiences of the investigators and represents an attempt to distinguish mild and marginal toxic symptoms (e.g., alopecia) from severe and medically important ones (e.g., cytopenias) (10). As shown below, it was designed to increase in proportion to the duration of toxic events, their intensity, and their clinical significance, and to decrease with the time on the protocol at which the toxic manifestations first appeared (i.e., if a toxic reaction occurred early in the course of treatment, this would result in a higher toxicity score than if the same reaction occurred only after a longer course of treatment). The duration of the toxic event was placed in the numerator of the toxicity score, in order to quantitatively emphasize persistent morbidities while minimizing the contribution of transitory morbidities and spurious abnormal laboratory values. The TS was calculated as follows:

$$TS = \Sigma\left[\frac{\text{(duration of toxic events [weeks])} \times \text{(intensity)} \times \text{(clinical severity factor)}}{\text{weeks on protocol}}\right]$$

where intensity = 1 (mild), 2 (moderate), or 3 (severe); and clinical severity factor = 1 (alopecia, nausea, pruritus, anorexia and/or general GI intolerance [pyrosis, cramps, etc.]), 2 (vomiting, diarrhea, stomatitis and/or rash), 3 (elevated liver enzyme levels and/or elevated serum creatinine level), or 4 (cytopenia, documented infections, and/or pulmonary toxicity).

Abnormal results on liver function tests were defined as transaminase and/or alkaline phosphatase values >2 times the baseline levels; cytopenia was defined as a WBC count <35,000/mm^3 or a platelet count <150,000/mm^3; elevated serum creatinine level was defined as >1.5 mg/dl; and pulmonary toxicity was defined as evidence of new interstitial pulmonary infiltrates compared with the baseline chest radiograph, with evidence of restrictive changes seen on pulmonary function tests (i.e., vital capacity <80% of predicted, normal expiratory flow rates, normal maximal voluntary ventilation, and carbon monoxide diffusing capacity <70% of normal [17]). Infection was defined as a documented viral, bacterial, or fungal infection that compromised the patient's condition significantly, required hospitalization, and/or required administration of systemic antibiotics or antifungal agents. Any abnormality in transaminase level, alkaline phosphatase level, WBC count, or platelet count, documented infection believed to be related to the MTX, or pulmonary toxicity was given an intensity score of 3 (severe).

Overall response to treatment was determined by a modification of the criteria developed by the Cooperative Systematic Studies of the Rheumatic Diseases group (18, 19), as follows. The score on the joint swelling index and the joint tenderness/pain index at visit 2 and/or visit 3 was compared with the score at visit 1. Marked improvement was defined as a ≥50% decrease in these scores, moderate improvement was defined as a 31–49% decrease in the index, no change was defined as a score remaining within 30% of the original value, and worsening was defined as a >30% increase in the score. Improvement and worsening in the physician or patient assessment of disease activity were defined as changes of at least 2 integers on the 5-point scales.

Table 2. Mean ± SD cumulative doses of methotrexate (MTX) and dietary intake of folate and vitamin B_{12}

	Visit 1	Visit 2	Visit 3
Cumulative MTX dose, mg			
Folate group	–	80 ± 21	183 ± 64
Placebo group	–	70 ± 25	150 ± 37
Dietary folate intake, μg/day			
Folate group	196 ± 112	274 ± 189	220 ± 135
Placebo group	206 ± 176	150 ± 90	131 ± 53
Dietary vitamin B_{12} intake, μg/day			
Folate group	2.1 ± 1.4	3.2 ± 2.6	2.0 ± 1.8
Placebo group	2.2 ± 1.0	1.5 ± 0.9	2.0 ± 1.2

Laboratory assessment. At visit 1, the complete blood cell count (CBC) including platelet count, Westergren ESR, liver enzyme levels (aspartate aminotransferase [AST] and alkaline phosphatase), RF titer, and serum creatinine level were determined. Followup CBC, creatinine studies, and liver function tests were performed at the discretion of each patient's rheumatologist and were generally repeated at each followup visit. Tests were performed more often (usually weekly) if an abnormal value was obtained.

Biochemical assessment. At visits 1, 2, and 3, blood was obtained for a vitamin screen (serum and red blood cell [RBC] folate, serum vitamin B_{12}, vitamin C, vitamin A, β-carotene, vitamin B_6, thiamine, and riboflavin) and for determination of the C_1 index, by methods previously described (20,21). The C_1 index measures the activity of a folate-dependent enzyme system in peripheral blood mononuclear cells by assaying the formation of serine from glycine and radiolabeled formate.

Statistical analysis. Either Student's *t*-test or analysis of variance followed by the least significant difference test was used to compare means of normally distributed data. The Wilcoxon signed rank test was used with C_1 index data. Spearman's rank correlation test and chi-square analysis were used when appropriate (22). *P* values less than or equal to 0.05 were considered significant.

RESULTS

Characteristics of the patient groups. Sixteen of the 32 patients were in the folate supplement group, and 16 were in the placebo group. Findings from pill counts and questioning suggested a high degree of compliance with the MTX and FA/placebo regimen.

Table 3. Patient response to methotrexate treatment*

Variable, patient group	No. of patients				
	Marked improvement	Moderate improvement	Marked or moderate improvement	No change	Worsening
Swelling index					
Visit 2					
Folate	6	3		5	0
Placebo	5	2		5	2
Visit 3					
Folate	7	3		4	1
Placebo	3	5		3	1
Pain/tenderness index					
Visit 2					
Folate	5	1		5	3
Placebo	7	1		2	4
Visit 3					
Folate	5	3		4	3
Placebo	7	2		0	3
Joint swelling count					
Visit 2					
Folate	3	5		4	2
Placebo	4	3		5	2
Visit 3					
Folate	8	2		3	2
Placebo	3	3		5	1
Joint pain/tenderness count					
Visit 2					
Folate	5	1		6	2
Placebo	7	0		2	5
Visit 3					
Folate	4	4		4	3
Placebo	6	1		2	3
Physician assessment					
Visit 2					
Folate			1	13	0
Placebo			0	11	1
Visit 3					
Folate			2	11	0
Placebo			0	10	0
Patient assessment					
Visit 2					
Folate			3	11	0
Placebo			4	8	2
Visit 3					
Folate			3	12	0
Placebo			2	10	0

* The initial study population consisted of 16 patients in the folate group and 16 in the placebo group. Numbers shown total less than 16 for each visit because patients dropped out of the study, missed visits, or the variable was not determined.

The mean duration of disease was longer in the placebo-treated group; other baseline demographic and clinical characteristics, including disease severity as measured by joint counts, anatomic stage, and C:MC ratio, were not different between the groups (Table 1). There were no statistically significant differences between the groups in baseline hemoglobin or hematocrit values, mean corpuscular volume (MCV), WBC counts, or AST, alkaline phosphatase, or serum creatinine levels. The groups did not differ in their initial mean serum and RBC folate levels. In addition, there was no significant difference between the groups in their mean dietary intake of folate or vitamin B_{12}, or in the cumulative MTX intake at any of the visits (Table 2). While there was a trend toward higher dietary folate consumption in the folate supplement group, it was not biologically important, given the much larger amount of FA that these patients received in the form

of the supplement. In addition, the patients' mean dietary intake of folate was below the recommended dietary allowance of 400 μg/day (23).

Efficacy. Table 3 shows a comparison between the folate supplement and the placebo groups in their degree of response to MTX treatment. Chi-square analysis failed to show any statistically significant difference between the 2 groups in the degree of improvement.

Toxicity and patient dropout. Fifteen patients (47%) experienced some form of toxicity; of these, 10 (67%) were in the placebo group, and 5 (33%) were in the folate supplement group. The toxic manifestations observed and the number of patients experiencing the reaction, in the placebo group and the folate supplement group, respectively, were as follows: nausea 8 and 5, elevations in liver enzyme levels 3 and 0, cytopenia 1 and 1, anorexia 2 and 0, alopecia 0 and 2, constipation/bloating 1 and 0, pyrosis 1 and 0, stomach cramps 0 and 1, stomatitis 0 and 1, and transiently elevated creatinine levels 1 and 0.

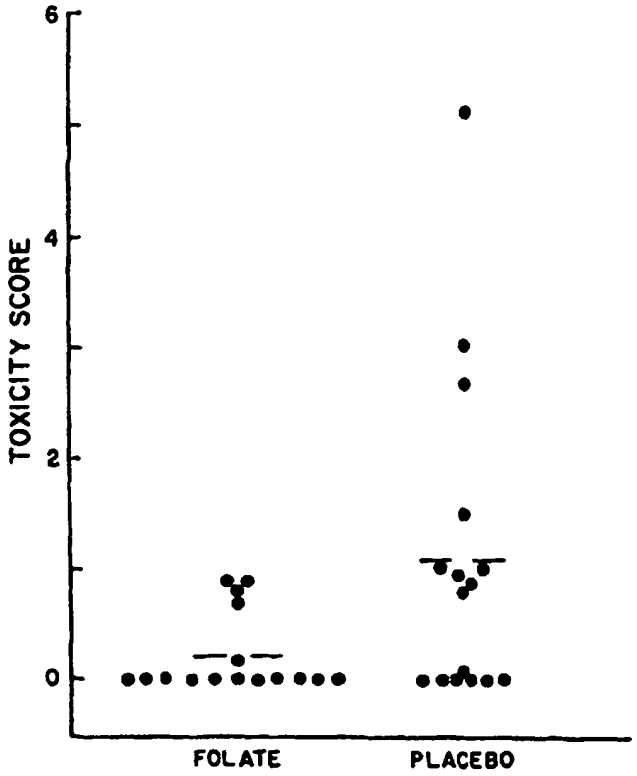

Figure 1. Toxicity scores in the folate supplement and placebo groups. Horizontal bars show the mean scores (0.21 and 1.06, respectively; $P = 0.027$). See Patients and Methods for explanation of toxicity score calculation.

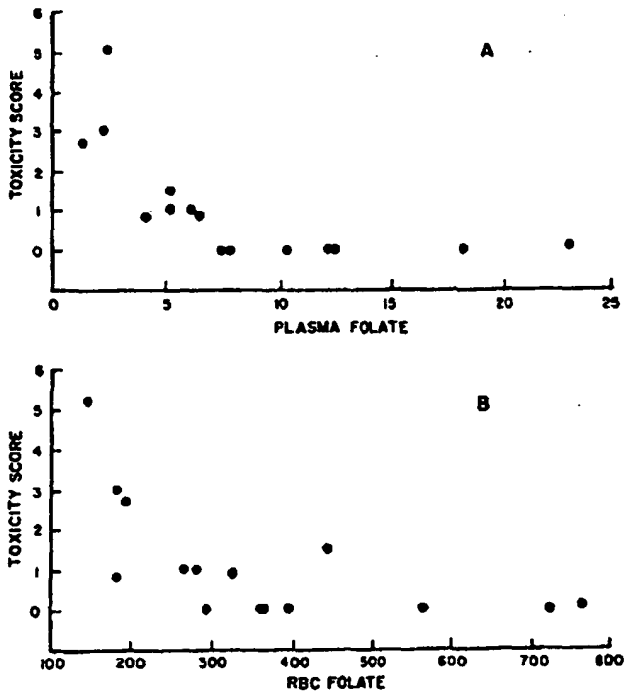

Figure 2. Correlation of toxicity score (TS; see Patients and Methods) with initial plasma (A) and red blood cell (RBC) (B) folate levels (ng/ml) in the placebo group. Spearman's rank correlation coefficient was -0.84 ($P < 0.01$) and -0.66 ($P < 0.01$) for plasma and RBC folate levels, respectively. Normal levels of plasma and RBC folate are ≥2 ng/ml and ≥140 ng/ml, respectively.

Five of the patients (16%) dropped out of the study before visit 3. Four of these patients were in the placebo group; their toxicity scores were 1.0, 2.67, 3.0, and 5.1. MTX had to be discontinued in these patients because of toxicity. The toxic reactions observed in the 4 patients were severe nausea, anorexia, constipation, persistently elevated liver enzyme levels (2 or more determinations 1 week apart), cytopenia, and elevated creatinine levels. Elevated creatinine levels were attributed to MTX toxicity since they normalized following discontinuation of the drug. In the 1 patient in the folate supplement group who was withdrawn from the study, MTX treatment was stopped, not because of toxicity, but in preparation for surgical joint replacement.

The mean toxicity score in the folate supplement group was significantly lower than that in the placebo group (Figure 1). Minor toxicity (i.e., toxicity score <0.2) or no toxicity occurred in 12 patients in the folate supplement group, whereas only 7 patients in the placebo group were in this category.

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